

DESIGN AND DEVELOPMENT OF REAL-TIME  
BIOELECTROCHEMICAL SENSOR FOR  
WASTEWATER MONITORING WITH INTEGRATED  
ALARM NOTIFICATION

RYAN YEO YOW ZHONG

UNIVERSITI KEBANGSAAN MALAYSIA

DESIGN AND DEVELOPMENT OF REAL-TIME BIOELECTROCHEMICAL  
SENSOR FOR WASTEWATER MONITORING WITH INTEGRATED ALARM  
NOTIFICATION

RYAN YEO YOW ZHONG

THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

FUEL CELL INSTITUTE  
UNIVERSITI KEBANGSAAN MALAYSIA  
BANGI

2026

REKAAN DAN PEMBANGUNAN SENSOR BIOELEKTROKIMIA MASA NYATA  
UNTUK PEMANTAUAN AIR SISA DENGAN NOTIFIKASI ALARM

RYAN YEO YOW ZHONG

TESIS YANG DIKEMUKAKAN UNTUK MEMPEROLEH IJAZAH  
KEDOKTORAN

INSTITUT SEL FUEL  
UNIVERSITI KEBANGSAAN MALAYSIA  
BANGI

2026

|                                                                                                                                                                                   |                                                                                                                                                        |                        |                                          |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|------------------------------------------|
|  <b>UNIVERSITI<br/>KEBANGSAAN<br/>MALAYSIA</b><br><i>The National University<br/>of Malaysia</i> | <b>UKM-SPKPPP-PT(PdP)-05-AK04-BO07</b>                                                                                                                 | <b>No. Semakan: 02</b> | <b>Tarikh Kuat Kuasa:<br/>27/01/2026</b> |
|                                                                                                                                                                                   | <b>BORANG DAN SENARAI SEMAK PENYERAHAN TESIS/DISERTASI<br/>SELEPAS PEMBETULAN<br/>FORM AND CHECKLIST OF AMENDED THESIS/DISSERTATION<br/>SUBMISSION</b> |                        |                                          |

|                                                                                                                                                                                                                                                                                                                                          |                                                                                                                            |                                                                                                                                                                                      |                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| <b>F. KELULUSAN DEKAN/PENGARAH / APPROVAL FROM DEAN/DIRECTOR</b>                                                                                                                                                                                                                                                                         |                                                                                                                            |                                                                                                                                                                                      |                                  |
| Tandatangan:<br><i>Signature</i>                                                                                                                                                                                                                                                                                                         |                                                                                                                            | Tarikh:<br><i>Date</i>                                                                                                                                                               |                                  |
| Nama dan Cap Rasmi:<br><i>Name and Official Stamp</i>                                                                                                                                                                                                                                                                                    |                                                                                                                            |                                                                                                                                                                                      |                                  |
| <b>G. PERAKUAN TESIS/ DISERTASI SARJANA / DOKTOR FALSAFAH<br/>(CERTIFICATION OF MASTER'S / DOCTORAL THESIS / DISSERTATION)</b>                                                                                                                                                                                                           |                                                                                                                            |                                                                                                                                                                                      |                                  |
| Nama penuh pengarang<br>(Author's Full Name)                                                                                                                                                                                                                                                                                             | Ryan Yeo Yow Zhong                                                                                                         |                                                                                                                                                                                      |                                  |
| No. Pendaftaran Pelajar<br>(Student's Registration No.)                                                                                                                                                                                                                                                                                  | P126713                                                                                                                    | Sesi Akademik<br>(Academic Session)                                                                                                                                                  | SEM 1 SESI AKADEMIK<br>2025/2026 |
| Tajuk Tesis / Disertasi<br>(Thesis / Dissertation Title)                                                                                                                                                                                                                                                                                 | Design And Development of Real-Time Bioelectrochemical Sensor for Wastewater Monitoring with Integrated Alarm Notification |                                                                                                                                                                                      |                                  |
| Semua tesis/disertasi dan penerbitan berkaitan hasil penyelidikan pelajar adalah tertakluk kepada Dasar Harta Intelek Universiti Kebangsaan Malaysia.<br><i>All theses/ dissertations and publications relating to the research work of a student are subject to the Intellectual Property Policy of Universiti Kebangsaan Malaysia.</i> |                                                                                                                            |                                                                                                                                                                                      |                                  |
| Saya mengaku bahawa tahap akses tesis/disertasi ini sebagai (*Tandakan ✓ dalam kotak bagi maklumat yang berkaitan; tandakan satu (1) kotak di bawah sahaja):<br><i>I hereby declare the level of access for this thesis/dissertation as follows (tick ✓ the relevant box below; tick only one option).</i>                               |                                                                                                                            |                                                                                                                                                                                      |                                  |
| <b>Pilihan Tahap Akses<br/>Access Level Selection</b>                                                                                                                                                                                                                                                                                    |                                                                                                                            | <b>Tafsiran Tahap Akses Tesis/Disertasi<br/>Definition of Thesis/Dissertation Access Level</b>                                                                                       |                                  |
| <input type="checkbox"/> <b>RAHSIA<br/>(CONFIDENTIAL)</b>                                                                                                                                                                                                                                                                                |                                                                                                                            | Mengandungi maklumat rahsia sepertimana yang diperuntukan bawah Akta Rahsia Rasmi 1972.<br><i>Contains classified information as stipulated under the Official Secrets Act 1972.</i> |                                  |

|                                                                                                                                                                                   |                                                                                                                                                              |                        |                                          |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|------------------------------------------|
|  <b>UNIVERSITI<br/>KEBANGSAAN<br/>MALAYSIA</b><br><i>The National University<br/>of Malaysia</i> | <b>UKM-SPKPPP-PT(PdP)-05-AK04-BO07</b>                                                                                                                       | <b>No. Semakan: 02</b> | <b>Tarikh Kuat Kuasa:<br/>27/01/2026</b> |
|                                                                                                                                                                                   | <b>BORANG DAN SENARAI SEMAK PENYERAHAN TESIS/DISERTASI<br/>SELEPAS PEMBETULAN</b><br><b>FORM AND CHECKLIST OF AMENDED THESIS/DISSERTATION<br/>SUBMISSION</b> |                        |                                          |

|                                     |                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------------------------------|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <b>TERHAD<br/>(RESTRICTED)</b>         | <p>Mengandungi maklumat yang hanya boleh diakses oleh pihak yang mempunyai kebenaran atau keperluan tertentu untuk mengetahuinya yang ditentukan oleh organisasi/ badan di mana penyelidikan dijalankan.</p> <p><i>Contains information that may only be accessed by parties with specific authorization or a defined need to know, as determined by the organization or body where the research is conducted.</i></p>                                                                                                                                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <b>AKSES TERBUKA<br/>(OPEN ACCESS)</b> | <p>Tesis/disertasi versi akhir dalam format PDF yang boleh diakses secara percuma secara dalam talian dan bebas dari isu hak cipta dan pelesenan</p> <p><i>The final version of the thesis/dissertation in PDF format that is freely accessible online and free from copyright and licensing issues.</i></p> <p>Kementerian Pendidikan Tinggi melalui surat bertarikh 27 Mac 2025 memutuskan bahawa kebenaran akses tesis / disertasi dalam borang serahan akses terbuka ditentukan oleh penyelia (bukan pelajar).</p> <p><i>The Ministry of Higher Education, through a letter dated 27 March 2025, has decided that permission for thesis / dissertation access in the open access submission form shall be determined by the supervisor (not the student).</i></p> |
| <input type="checkbox"/>            | <b>EMBARGO</b>                         | <p>Tempoh masa di mana akses kepada tesis/disertasi disekat (ditutup) selama dua (2) tahun atas sebab-sebab tertentu sebagai contoh kerahsiaan atau hak cipta.</p> <p>Tesis/disertasi akan berstatus akses terbuka selepas tamat tempoh embargo.</p> <p><i>The period during which access to the thesis/dissertation is restricted for two (2) years due to specific reasons, such as confidentiality or copyright. The thesis/dissertation will be granted open-access status upon the expiry of the embargo period.</i></p>                                                                                                                                                                                                                                         |

| <b>PENGESAHAN PELAJAR (STUDENT VERIFICATION)</b>                                                                                                           | <b>PENGESAHAN PENYELIA (SUPERVISOR VERIFICATION)</b>                                                                                                                                                                                                                                                                                                                                                                                        |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <br><b>TANDATANGAN PELAJAR<br/>(STUDENT'S SIGNATURE)</b>                | <br><b>TANDATANGAN PENYELIA / Pengerusi JK SISWAZAH<br/>(SUPERVISOR'S / CHAIRPERSON SUPERVISION<br/>COMMITTEE SIGNATURE)</b>                                                                                                                                                                                                                           |
| <p>Nama Pelajar (Student name):<br/>No. Pendaftaran Pelajar<br/>(Student's Registration No.)</p> <p>P126713</p> <p>Tarikh:<br/>(Date)</p> <p>29/1/2026</p> | <p>Nama Penyelia /<br/>Pengerusi JK Siswazah :<br/>(Supervisor's / Chairperson<br/>Supervision Committee<br/>Name)</p> <p><br/> <b>DR. ANG WEI LUN</b><br/> Pensyarah Kanan<br/> Pusat Penyelidikan Teknologi Proses Mampar<br/> Fakulti Kejuruteraan dan Alam Bina<br/> Universiti Kebangsaan Malaysia</p> <p>Tarikh:<br/>(Date)</p> <p>30/1/2026</p> |

### **DECLARATION**

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

04 February 2026

RYAN YEO YOW ZHONG  
P126713

## ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to the Fuel Cell Institute (SELFUEL) of Universiti Kebangsaan Malaysia (UKM) for providing me knowledge and facilities towards the completion of my studies and thesis.

I am extremely grateful to my supervisors Dr. Ang Wei Lun and Dr. Lim Swee Su for giving me support and guidance in the laboratory as well as conducting experiments at real-world industrial settings. I would like to thank Prof. Dr. Eileen Hao Yu and Dr. Wong Wai Yin for giving me new inputs during every progress meeting. Next, I would like to thank Dr. Mimi Hani Abu Bakar and Dr. Manal Ismail for constantly checking in and making sure I am on track with my progress and experimental works. Next, I am thankful for Mr. Ahmad Syakir Saad and Mdm. Hafizatul Izzati Badrul Zaman for assisting with the preparation and submission of the thesis.

Last but not least, I would love to offer my greatest thanks to my parents for their continuous support since the beginning of my studies at UKM. They have been supporting me financially and emotionally throughout my completion of my thesis.

## ABSTRAK

Sensor bioelektrokimia merupakan sejenis biosensor yang menggunakan metabolisme mikrob untuk menukar isyarat kimia daripada analit kepada isyarat elektrik yang boleh diukur. Pada masa ini, kaedah pemantauan air sisa konvensional masih bergantung kepada analisis kimia yang memakan masa, berkos tinggi, dan lazimnya dilakukan di luar tapak. Justeru, sensor bioelektrokimia merupakan calon terbaik untuk menangani isu ini kerana keupayaannya memberikan maklum balas pantas terhadap kehadiran pencemar melalui penghasilan elektrik. Dalam kajian ini, satu sensor elektrokimia mikrob dua elektrod (MESe) dengan anod yang diubah suai (salutan serbuk karbon, pengoksidaan api, pelunturan asid) telah dibangunkan dan diuji terhadap pelbagai jenis pencemar. MESe dengan anod bersalut serbuk karbon menunjukkan ketepatan tertinggi ( $R^2$ ) dalam ujian pencemar, dengan julat antara 0.5891 hingga 0.9754 apabila dikorelasi dengan data Keperluan Oksigen Kimia (COD). Walau bagaimanapun, MESe sering mengalami isu isyarat akibat halangan antara anod dan katod. Bagi mengatasi masalah ini, reka bentuk jenis tiub bagi MFCSes dengan katod udara yang diubah suai (kadar salutan PtC antara 0.5 – 2.0 mg cm<sup>-2</sup>) telah dicadangkan bagi meningkatkan prestasi MESe. Reka bentuk ini menunjukkan ketepatan yang lebih tinggi walaupun tanpa bekalan kuasa luaran. MFCSes dengan salutan PtC sebanyak 1.5 mg cm<sup>-2</sup> mencatatkan ketepatan tertinggi, antara 0.8248 hingga 0.9655 apabila dikorelasi dengan data COD. Satu platform berasaskan awan dengan sistem notifikasi amaran turut dibangunkan bagi membolehkan pemantauan dalam talian secara masa nyata. Dua model matematik (pengesanan anomali dan pengesanan titik perubahan) telah diintegrasikan dengan sistem notifikasi ini bagi membolehkan pemantauan tanpa pengawasan manusia. MFCSes bersama sistem notifikasi amaran ini telah berjaya diuji dan digunakan di sebuah loji rawatan air sisa perbandaran sebenar. Sistem yang menyeluruh ini membuktikan keupayaannya untuk mengesan serta menghantar amaran bagi pelbagai senario operasi, termasuk kemasukan air mentah dan cair, gangguan mekanikal, serta bunyi persekitaran.

## ABSTRACT

Bioelectrochemical sensors are a class of biosensors that use microbial metabolism to convert chemical signals from an analyte into a measurable electrical signal. Currently, conventional wastewater monitoring methods rely on chemical analysis, which are time consuming, costly, and typically performed off-site. Hence, bioelectrochemical sensors are a perfect candidate to address this issue due to their ability to provide rapid feedback on the presence of pollutants through electricity production. In this work, a two-electrode microbial electrochemical sensor (MESe) with modified anodes (carbon powder-coated, flame oxidised, acid bleached) were developed and tested against multiple pollutants. MESe with carbon powder-coated anode achieved the highest accuracy ( $R^2$ ) in the pollutant test ranging from 0.5891 to 0.9754 when correlated with chemical oxygen demand (COD) data. However, MESe often encountered signal issues due to blockages between the anode and cathode. To address this, a tubular-type MFCSe with modified air-cathodes ( $0.5 - 2.0 \text{ mg cm}^{-2}$  PtC loading rate) was proposed to improve the MESe design, which demonstrated higher accuracy even without external power supply. MFCSe with  $1.5 \text{ mg cm}^{-2}$  PtC obtained the highest accuracy ranging from 0.8248 to 0.9655 when correlated with COD data. Cloud platform with alarm notification system was established to allow real-time online monitoring and two mathematical models (anomaly detection and change point detection) were integrated with the notification system to allow unmanned supervision. The MFCSe, along with its alarm notification system, was successfully deployed at an actual municipal wastewater treatment facility. This comprehensive system demonstrated its capability to detect and send an alarm for various operational scenarios, including fresh and diluted influent, mechanical disturbances, and surrounding noises.

## TABLE OF CONTENTS

|                                                                      | <b>Page</b> |
|----------------------------------------------------------------------|-------------|
| <b>DECLARATION</b>                                                   | iii         |
| <b>ACKNOWLEDGEMENT</b>                                               | iv          |
| <b>ABSTRAK</b>                                                       | v           |
| <b>ABSTRACT</b>                                                      | vi          |
| <b>TABLE OF CONTENTS</b>                                             | vii         |
| <b>LIST OF TABLES</b>                                                | xi          |
| <b>LIST OF FIGURES</b>                                               | xii         |
| <b>LIST OF ABBREVIATIONS</b>                                         | xvi         |
| <b>LIST OF NOMENCLATURE</b>                                          | xviii       |
| <br><b>CHAPTER I      INTRODUCTION</b>                               |             |
| 1.1                  Research Background                             | 1           |
| 1.2                  Problem Statement                               | 4           |
| 1.3                  Research Hypotheses                             | 6           |
| 1.4                  Research Objectives                             | 7           |
| 1.5                  Research Scope                                  | 8           |
| 1.6                  Contribution to the Body of Knowledge           | 8           |
| <br><b>CHAPTER II      LITERATURE REVIEW</b>                         |             |
| 2.1                  Water Quality Monitoring                        | 10          |
| 2.1.1      Importance of water quality monitoring                    | 10          |
| 2.1.2      Conventional water quality monitoring methods             | 11          |
| 2.1.3      Background of key pollutants in wastewater monitoring     | 14          |
| 2.1.4      Regulatory standards for wastewater discharge in Malaysia | 17          |
| 2.2                  Bioelectrochemical System (BES)                 | 18          |
| 2.2.1      BES mechanism                                             | 19          |

|                                    |                                                                   |    |
|------------------------------------|-------------------------------------------------------------------|----|
| 2.2.2                              | Electron transfer mechanisms                                      | 20 |
| 2.2.3                              | Bioelectrochemical system (BES)-based sensor                      | 24 |
| 2.2.4                              | Existing MESe with stainless steel electrodes                     | 24 |
| 2.2.5                              | Existing MFCSe with modified air-cathodes                         | 27 |
| 2.3                                | BES Anode and Cathode Materials                                   | 28 |
| 2.3.1                              | Anode materials                                                   | 28 |
| 2.3.2                              | Cathode materials                                                 | 31 |
| 2.4                                | Internet of Things (IoT) in Sensor Technology                     | 32 |
| 2.4.1                              | Cloud data streaming for real-time monitoring                     | 33 |
| 2.4.2                              | Real-time notification systems for instant alerts                 | 34 |
| 2.5                                | Mathematical Models in Sensor Technology                          | 35 |
| 2.5.1                              | Anomaly detection: Identifying outliers                           | 35 |
| 2.5.2                              | Change point detection: Identifying onset of change               | 36 |
| 2.6                                | Summary of Research Gap                                           | 37 |
| <br><b>CHAPTER III METHODOLOGY</b> |                                                                   |    |
| 3.1                                | Introduction                                                      | 39 |
| 3.2                                | List of Chemicals and Materials                                   | 41 |
| 3.3                                | Instrumentations                                                  | 42 |
| 3.4                                | Design and Development of Microbial Electrochemical Sensor (MESe) | 43 |
| 3.4.1                              | Design and construction of MESe setup                             | 43 |
| 3.4.2                              | Design and fabrication of MESe electrodes                         | 44 |
| 3.4.3                              | Assembly of electrodes and installation of MESe                   | 45 |
| 3.4.4                              | Physicochemical characterization of electrodes                    | 45 |
| 3.4.5                              | Electrochemical characterization of electrodes                    | 46 |
| 3.4.6                              | Startup, operation, and enrichment                                | 46 |
| 3.4.7                              | Pollutants testing                                                | 47 |
| 3.4.8                              | Liquid sample analysis                                            | 48 |

|                   |                                                              |     |
|-------------------|--------------------------------------------------------------|-----|
| 3.5               | Design and Development of Microbial Fuel Cell Sensor (MFCSe) | 48  |
| 3.5.1             | Design and construction of MFCSe setup                       | 48  |
| 3.5.2             | Design and fabrication of MFCSe electrodes                   | 49  |
| 3.5.3             | Assembly of electrodes and installation of MFCSe             | 50  |
| 3.6               | Integration of Alarm Notification                            | 51  |
| 3.7               | MFCSe for Actual Municipal Wastewater Treatment Conditions   | 52  |
| 3.7.1             | Fabrication of pilot scale MFCSe for site                    | 52  |
| 3.7.2             | Installation and application                                 | 53  |
| <b>CHAPTER IV</b> | <b>RESULTS AND DISCUSSION</b>                                |     |
| 4.1               | Introduction                                                 | 55  |
| 4.2               | Microbial Electrochemical Sensor (MESe)                      | 55  |
| 4.2.1             | Physicochemical analysis                                     | 55  |
| 4.2.2             | Electrochemical properties                                   | 64  |
| 4.2.3             | Pollutants testing                                           | 70  |
| 4.2.4             | Correlation data                                             | 78  |
| 4.2.5             | Summary                                                      | 81  |
| 4.3               | Microbial Fuel Cell Sensor (MFCSe)                           | 82  |
| 4.3.1             | Physicochemical analysis                                     | 83  |
| 4.3.2             | Electrochemical properties                                   | 91  |
| 4.3.3             | Enrichment                                                   | 93  |
| 4.3.4             | Pollutants testing                                           | 95  |
| 4.3.5             | Correlation data                                             | 108 |
| 4.3.6             | Microbial community analysis                                 | 112 |
| 4.3.7             | Summary                                                      | 114 |
| 4.4               | Alarm Notification System with Internet of Things (IoT)      | 115 |
| 4.4.1             | Performance of MFCSe integrated with mathematical models     | 115 |
| 4.4.2             | Summary                                                      | 117 |
| 4.5               | Actual Municipal Wastewater Quality Detection                | 118 |
| 4.5.1             | Detection of actual municipal wastewater                     | 118 |
| 4.5.2             | Sensitivity                                                  | 120 |

|                   |                                         |     |
|-------------------|-----------------------------------------|-----|
| <b>CHAPTER V</b>  | <b>CONCLUSION AND FUTURE DIRECTIONS</b> |     |
| 5.1               | Conclusion                              | 122 |
| 5.2               | Future Directions                       | 124 |
| <b>REFERENCES</b> |                                         | 126 |
| <b>APPENDICES</b> |                                         |     |
| Appendix A        | List of Publications                    | 147 |



## LIST OF TABLES

| Table No. |                                                                                                                                                                    | Page |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Table 2.1 | Acceptable conditions for discharge of industrial effluent or mixed effluent of standards A and B ("Environment Quality (Industrial Effluent) Regulations 2009."). | 17   |
| Table 2.2 | A summary of stainless steel anodes and their modifications in MESes.                                                                                              | 25   |
| Table 2.3 | A summary of air-cathodes and their modifications in MFCSeS.                                                                                                       | 27   |
| Table 3.1 | List of materials and chemicals used in this study.                                                                                                                | 41   |
| Table 3.2 | List of instruments used in this study.                                                                                                                            | 42   |
| Table 3.3 | Materials for water circulation setup construction.                                                                                                                | 43   |
| Table 3.4 | Pollutant testing sequences and their purpose.                                                                                                                     | 47   |
| Table 4.1 | EDX values of the modified MESe electrodes.                                                                                                                        | 59   |
| Table 4.2 | AFM line profiling parameters.                                                                                                                                     | 62   |

## LIST OF FIGURES

| Figure No. |                                                                                                                                                                                       | Page |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Figure 2.1 | Mechanism of Bioelectrochemical systems (BES).                                                                                                                                        | 20   |
| Figure 2.2 | Scanning electron microscope (SEM) images of <i>Shewanella Oneidensis</i> MR-1 biofilm (a) developed on anode carbon felt fibers and (b) with nanowires.                              | 22   |
| Figure 3.1 | Research flowchart of this study.                                                                                                                                                     | 40   |
| Figure 3.2 | Schematic illustration of the MESe setup.                                                                                                                                             | 43   |
| Figure 3.3 | Electrode design of MESe (a) schematic drawing and (b) actual electrode coated with catalysts (from left to right: control, carbon-coated, flame oxidised, and acid bleached).        | 44   |
| Figure 3.4 | MESe electrodes assembly (a) schematic drawing and (b) actual assembled MESe.                                                                                                         | 45   |
| Figure 3.5 | Schematic illustration of (a) MFCSe setup, (b) side view and (c) top view of the pipe openings for the sensor.                                                                        | 49   |
| Figure 3.6 | MFCSe electrodes assembly using a MEA configuration.                                                                                                                                  | 50   |
| Figure 3.7 | (a) Design and (b) Construction of MFCSe biosensor for actual municipal wastewater quality detection.                                                                                 | 53   |
| Figure 3.8 | (a) On-site MFCSe installation, (b) Assembled MFCSe, (c) Data acquisition system, and (d) cable connection.                                                                           | 54   |
| Figure 4.1 | SEM images at 1000x magnification for (a) control, (b) carbon-coated, (c) flame oxidised, and (d) acid bleached MESe electrodes.                                                      | 56   |
| Figure 4.2 | EDX elemental mapping at 500x magnification for (a) control, (b) carbon-coated, (c) flame oxidised, and (d) acid bleached MESe electrodes.                                            | 59   |
| Figure 4.3 | Topography imaging of the electrode surfaces (left images are 3D; right images are 2D & histogram): (a) Control, (b) carbon powder coated, (c) flame oxidised, and (d) acid bleached. | 61   |
| Figure 4.4 | Raman spectrogram of (a) control, (b) carbon-coated, (c) flame oxidised, and (d) acid bleached MESe electrodes.                                                                       | 63   |

|             |                                                                                                                                                                                                                                              |    |
|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 4.5  | (a) Cell and (b) anode resistances of MESe with modified electrodes, where $R_p$ and $R_s$ represents polarization and solution resistances, respectively.                                                                                   | 65 |
| Figure 4.6  | Cyclic voltammogram of MESe with modified electrodes.                                                                                                                                                                                        | 69 |
| Figure 4.7  | Signal profile and COD during pollutants testing for sodium acetate.                                                                                                                                                                         | 72 |
| Figure 4.8  | Signal profile and COD during pollutants testing for ethanol.                                                                                                                                                                                | 73 |
| Figure 4.9  | Signal profile and COD during pollutants testing for POME.                                                                                                                                                                                   | 74 |
| Figure 4.10 | Signal profile and COD during pollutants testing for thinner.                                                                                                                                                                                | 77 |
| Figure 4.11 | Correlation data between current density and COD values for (a) sodium acetate, (b) ethanol, (c) thinner, and (d) POME.                                                                                                                      | 80 |
| Figure 4.12 | FESEM images of biofilm formation on plain carbon cloth anode at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.                                                                                                   | 84 |
| Figure 4.13 | FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at 0.5 mg cm <sup>-2</sup> loading rate of PtC on carbon cloth air-cathode (before pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum. | 87 |
| Figure 4.14 | FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at 2.0 mg cm <sup>-2</sup> loading rate of PtC on carbon cloth air-cathode (before pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum. | 88 |
| Figure 4.15 | FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at 0.5 mg cm <sup>-2</sup> loading rate of PtC on carbon cloth air-cathode (post-pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.   | 90 |
| Figure 4.16 | FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at 2.0 mg cm <sup>-2</sup> loading rate of PtC on carbon cloth air-cathode (post-pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.   | 91 |

|             |                                                                                                                                                                                                                   |     |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 4.17 | Average cell resistances of MFCSe with (a) different air-cathode materials and (b) PTFE/CB/PtC air-cathode with different PtC loading rates.                                                                      | 93  |
| Figure 4.18 | Enrichment data.                                                                                                                                                                                                  | 95  |
| Figure 4.19 | (a) Signal profile and COD during pollutants testing for 4-Nitrophenol (4NP), average chemical analysis data (b) Total nitrogen (TN), (c) Injection concentration, (d) conductivity, (e) pH, and (f) temperature. | 98  |
| Figure 4.20 | (a) Signal profile and COD during pollutants testing for malathion, average chemical analysis data (b) Total phosphate, (c) conductivity, (d) pH, and (e) temperature.                                            | 102 |
| Figure 4.21 | (a) Signal profile and COD during pollutants testing for POME, average chemical analysis data (b) conductivity, (c) pH, and (d) temperature.                                                                      | 105 |
| Figure 4.22 | (a) Signal profile and injection concentration during pollutant testing for methyl orange, average chemical analysis data (b) conductivity, (c) pH, and (d) temperature.                                          | 108 |
| Figure 4.23 | Linear correlation graphs depicting the averaged current density in responses to COD during 4NP pollutant test.                                                                                                   | 109 |
| Figure 4.24 | Linear correlation graphs depicting the averaged current density in responses to COD during malathion pollutant test.                                                                                             | 110 |
| Figure 4.25 | Linear correlation graphs depicting the averaged current density in responses to COD during POME pollutant test.                                                                                                  | 111 |
| Figure 4.26 | (a) Linear and (b) Non-linear correlation graphs depicting the averaged current density in responses to MO concentration during MO pollutant test.                                                                | 112 |
| Figure 4.27 | 16s Amplicon analysis of microbial community.                                                                                                                                                                     | 114 |
| Figure 4.28 | (a) Rate of change (current density per minute) and (b) noise.                                                                                                                                                    | 116 |
| Figure 4.29 | (a) Change point detection and (b) anomaly detection.                                                                                                                                                             | 117 |
| Figure 4.30 | Notification email sent to the user when an alert is triggered.                                                                                                                                                   | 117 |

|             |                                                                                                                                           |     |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 4.31 | Data Collection during (a) Microbial Enrichment Process. (b) Monitoring Operation after microbial growth.                                 | 119 |
| Figure 4.32 | Mechanical disturbances and noise.                                                                                                        | 120 |
| Figure 4.33 | (a) Complete On-site data from biofilm development phase to stable phase and (b) biosensor's sensitivity range based on IWRC sewage tank. | 121 |



**LIST OF ABBREVIATIONS**

|                 |                                              |
|-----------------|----------------------------------------------|
| 2e <sup>-</sup> | 2-electron                                   |
| 4e <sup>-</sup> | 4-electron                                   |
| 4NP             | 4-nitrophenol                                |
| AAS             | Atomic absorption spectroscopy               |
| AFM             | Atomic force microscopy                      |
| BES             | Bioelectrochemical system                    |
| BOD             | Biochemical oxygen demand                    |
| CAD             | Computer aided design                        |
| CB              | Carbon black                                 |
| CC              | Carbon cloth                                 |
| CEM             | Cation exchange membrane                     |
| COD             | Chemical oxygen demand                       |
| CP              | Carbon paper                                 |
| CV              | Cyclic voltammetry                           |
| DC              | Direct current                               |
| DET             | Direct electron transfer                     |
| DO              | Dissolved oxygen                             |
| DOE             | Department of Environment                    |
| EDX             | Energy-dispersive X-ray                      |
| EET             | Extracellular electron transfer              |
| EIS             | Electrochemical impedance spectroscopy       |
| EPS             | Extracellular polymeric substances           |
| FESEM           | Field emission scanning electron microscope  |
| GO              | Graphene oxide                               |
| IC              | Ion chromatography                           |
| ICP-MS          | Inductively coupled plasma mass spectroscopy |
| IET             | Indirect electron transfer                   |

|       |                                  |
|-------|----------------------------------|
| IoT   | Internet of things               |
| LOD   | Limit-of-detection               |
| MEA   | Membrane-electrode assembly      |
| MESe  | Microbial electrochemical sensor |
| MFCSe | Microbial fuel cell sensor       |
| MO    | Methyl orange                    |
| MPN   | Most probable number             |
| ORR   | Oxygen reduction reaction        |
| PANI  | Polyaniline                      |
| POME  | Palm oil mill effluent           |
| PTFE  | Polytetrafluoroethylene          |
| PVA   | Polyvinyl alcohol                |
| RoC   | Rate-of-change                   |
| SDG   | Sustainable development goal     |
| SEM   | Scanning electron microscope     |
| TDS   | Total dissolved solids           |
| TSS   | Total suspended solids           |
| WHO   | World health organization        |
| WWTP  | Wastewater treatment plant       |

**LIST OF NOMENCLATURE**

|                                                                |                        |
|----------------------------------------------------------------|------------------------|
| C                                                              | Carbon                 |
| C <sub>2</sub> H <sub>5</sub> OH                               | Ethyl alcohol          |
| C <sub>3</sub> H <sub>7</sub> OH                               | Isopropyl alcohol      |
| CH <sub>3</sub> COO <sup>-</sup>                               | Acetate ion            |
| CH <sub>3</sub> COONa                                          | Sodium acetate         |
| C <sub>6</sub> H <sub>5</sub> NH <sub>2</sub>                  | Aniline                |
| C <sub>6</sub> H <sub>5</sub> NO <sub>3</sub>                  | 4-nitrophenol          |
| C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                  | Glucose                |
| C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub> | Malathion              |
| CO <sub>2</sub>                                                | Carbon dioxide         |
| Fe <sub>3</sub> O <sub>4</sub>                                 | Magnetite              |
| H <sup>+</sup>                                                 | Hydrogen ion           |
| HCl                                                            | Hydrochloric acid      |
| HCO <sub>3</sub> <sup>-</sup>                                  | Hydrogen carbonate ion |
| HNO <sub>3</sub>                                               | Nitric acid            |
| H <sub>2</sub> O                                               | Water                  |
| KOH                                                            | Potassium hydroxide    |
| NaOH                                                           | Sodium hydroxide       |
| NH <sub>4</sub> Cl                                             | Ammonium chloride      |
| (NH <sub>4</sub> ) <sub>2</sub> S <sub>2</sub> O <sub>8</sub>  | Ammonium persulfate    |
| Pt/C                                                           | Platinum on carbon     |

## CHAPTER I

### INTRODUCTION

#### 1.1 RESEARCH BACKGROUND

Water is an essential resource for human survival, economic activities, and environmental sustainability. However, water pollution has become an increasing global concern due to population growth, rapid industrialization, and agricultural expansion (Ahtasham Iqbal et al. 2024). Therefore, effective water quality monitoring is crucial for detecting pollution and illegal discharges to ensure the safety of aquatic ecosystems and public health in the face of increasing contamination (Neale et al. 2023). For wastewater treatment plants (WWTPs), the continuous evaluation of influent and effluent water quality is vital for several reasons. Apart from protecting aquatic ecosystems and public health, water quality monitoring ensures the proper functioning and longevity of treatment systems, supports regulatory compliance, and protects investments in expensive infrastructure. Inadequate or delayed detection of water quality issues may result in equipment malfunction, reduced treatment efficiency, environmental violations, and loss of public trust. For instance, in 2019, toxic chemical waste was illegally discharged into the Kim Kim River near an industrial area in Pasir Gudang, Johor. The incident affected over 4 000 people with most of them being school children. Many were rushed for medical treatment after experiencing breathing difficulties, nausea and vomiting due to exposure to hazardous gases emitted from Kim Kim River nearby their schools (Ibrahim et al. 2021; Ismail et al. 2020).

Conventional wastewater monitoring techniques used by treatment plants are essential for assessing water quality and ensuring regulatory compliance. These

methods typically involve periodic sampling and laboratory analysis to measure key physicochemical and biological parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), nitrogen compounds, and microbial contaminants. Common analytical techniques include spectrophotometry, chromatography, and culture-based microbiological assays. While accurate, these methods are generally applied to discrete samples collected at interval and they often rely on centralized laboratories, trained personnel, and specialized instruments (Yeo et al. 2023). Episodic discharges from industrial facilities, stormwater runoff, or accidental spills can occur between sampling intervals, leading to an incomplete assessment of water quality dynamics (Matesun et al. 2024). Given these challenges, there is a growing need for advanced, real-time monitoring technologies to enhance wastewater surveillance and treatment efficiency (Moretti et al. 2024; Syrmos et al. 2023).

In recent years, biosensor technology has emerged as a promising alternative for more responsive and accessible wastewater monitoring. Biosensors are analytical devices that combine a biological recognition with a transducer to convert biochemical interactions into quantifiable electrical, thermal, or optical signals (Srivastava et al. 2022). These devices are particularly attractive for on-site monitoring due to their simplicity, rapid response, lower operational cost, and potential for miniaturization. In environmental applications, biosensors have been used to detect various contaminants such as heavy metals (Velusamy et al. 2022), pesticides (Kalyani et al. 2021), pharmaceuticals (Campaña et al. 2019), and organic pollutants (Liu et al. 2022). For instance, enzymatic biosensors have been applied to detect phenolic compounds, while whole-cell biosensors have been engineered for toxicity assessment (Ma et al. 2020). Despite their promising performances, many conventional biosensors still require external chemical reagents, have limited lifespans, and are sensitive to matrix interference from complex wastewater samples (Yoo et al. 2020). These limitations have prompted the development of more robust and self-sustaining biosensing platforms based on electroactive microorganisms.

One of the most promising advancements in this area is bioelectrochemical system (BES)-based sensors, which exploits the metabolic activity of electrogenic bacteria to generate a direct electrical output in response to substrate availability or

toxicity (Sevda et al. 2020). Types of BES-based sensors including microbial electrochemical sensor (MESe) and microbial fuel cell sensor (MFCSe). The key distinction between MESe and MFCSe lies in their mode of operation and energy requirements. MESe typically operate under controlled electrochemical conditions, often using an externally applied potential to enhance electron transfer and improve sensitivity toward specific analytes. This configuration allows greater control over sensor response but requires external power input. In contrast, MFCSe function as self-powered devices, generating electrical signals solely from microbial metabolism without the need for an external energy source (Simoska et al. 2021; Sonawane et al. 2020). This self-sustaining nature makes MFCSe particularly attractive for long-term, remote, and decentralized monitoring applications in wastewater treatment facilities. Both sensor types have demonstrated the ability to detect changes in organic loading as well as toxic shock events, offering rapid feedback compared to conventional analytical methods.

Beyond sensor configuration, reactor design plays a crucial role in determining BES sensor performance, stability, and suitability for real-world deployment (Mittal et al. 2022). Among various geometries investigated, tubular-type BES sensors have gained increasing attention for wastewater monitoring applications. The tubular design offers several inherent advantages over planar or box-type configurations, including a higher surface-area-to-volume ratio, improved hydraulic flow characteristics, and more uniform substrate distribution across the biofilm (Wang et al. 2023), which promotes the growth of biofilm for bioelectrochemical operations. These features enhance mass transfer, and reduce the risk of dead zones or clogging during continuous operation (Zhang et al. 2024). Furthermore, tubular reactors are easier to integrate directly into wastewater pipelines or channels, making them well suited for in-situ monitoring in WWTPs environments.

The application of BES-based sensors in real-world municipal wastewater treatment plants represents a crucial step toward validating their practicality, reliability, and long-term performance under complex and variable operating conditions. Unlike controlled laboratory environments, actual field deployment must contend with fluctuating temperatures, inconsistent flow rates, and mixed pollutant loads, all of which

can affect microbial activity and sensor responsiveness. Their ability to detect both gradual pollutant buildup and sudden toxic shock events in real time enhances their value as early warning tools for wastewater treatment operations. When combined with Internet of Things (IoT) platforms and automated alarm systems, BES-based sensors enable remote monitoring and facilitate timely decision-making. Evaluating the performance of MESe and MFCSe in a functioning municipal treatment facility offers critical insights into their real-world capabilities and supports the transition toward more intelligent and decentralized water quality monitoring systems.

## 1.2 PROBLEM STATEMENT

The assessment of wastewater quality is an integral part of the operation of wastewater treatment plants (WWTPs). Among conventional wastewater monitoring techniques, most chemical analyses for wastewater quality are performed off-site, causing long-term delays for treatment plants. For instance, COD test results can be obtained within a few hours, while BOD analysis requires five to seven days depending on the trained personnel. These methods are unable to reflect the instantaneous state of incoming wastewater or detect severe pollution events in real-time. Similarly, culture-based enumeration methods for identifying harmful biological contaminants are also time-consuming. Apart from the aforementioned traditional water quality analysis methods, the current routine practiced by treatment plant employees involves manually observing and identifying the physical appearance of influents based on unusual color or smell by relying on their accumulated experience. However, this approach is highly subjective and varies from one individual to another. Such inaccurate and unreliable practices may lead to increased downtime of WWTPs, reducing treatment efficiency and incurring unnecessary expenses. By exploiting this loophole, illegal discharges continue to occur and more hazardous toxicants are released into the sewerage system forcing the WWTPs to shut down, which directly impacts consumers with water disruptions. Hence, a real-time wastewater quality monitoring technology with high sustainability and reasonable cost is essential to address this issue.

Specifically, to address this gap, a sensor technology based on bioelectrochemistry is a perfect candidate for this application. By utilizing electrogenic

microorganisms as biocatalysts, the oxidation of organic and inorganic components generates an electrical signal that can be recorded and converted into graphical data. Microbial electrochemical sensors (MESes) have emerged as a promising technology because of their spontaneous, real-time response to water quality variations and pollutants. However, conventional two-electrode MESes submerged in electrolyte require an external power source to drive the oxygen reduction reaction (ORR) at the cathode. The ORR is a key reaction and one of the most energy-demanding processes in bioelectrochemical systems (BESes), often becoming a bottleneck in sensor deployment. Furthermore, these submerged cathodes often suffer from clogging or biofouling, especially in high-solids or complex wastewater streams. This issue reduces sensor sensitivity over time and requires frequent maintenance, leading to increased operating costs. Although some research has suggested the use of air-cathodes as an alternative, feasibility studies under realistic wastewater conditions have been limited. Recent studies point toward the use of tubular microbial fuel cell sensors (MFCSe), where exposing the cathode to the atmospheric environment allows direct utilization of atmospheric oxygen molecules, eliminating the need for an external power supply and mitigating clogging concerns. This design enables continuous operation with minimal energy input and low maintenance requirements.

Additionally, wastewater treatment processes are becoming increasingly complex due to rapid population growth, higher influent volumes, and booming industrialization. Manual inspections are labor-intensive and costly as more employees must be stationed at different locations within the WWTP. Communication among these employees is greatly restricted, delaying decision-making. Serious damage to plant equipment can occur due to prolonged exposure to abnormal conditions or highly toxic contaminants. Hence, a practical and automated alarm notification system is needed to alert operators instantly of abnormal readings or potential equipment failures. Integrating an Internet of Things (IoT)-enabled monitoring system with mathematical models for limit-of-detection and rate-of-change (RoC) thresholding can provide real-time data processing and automatic notifications, allowing quick intervention. This approach will minimize human error, reduce downtime and maintenance costs, and enhance the overall resilience and responsiveness of the treatment plant.

Finally, although some lab-scale biosensors have demonstrated promising sensitivity and response under controlled conditions, their behavior in real-world WWTP environments with mixed pollutants, fluctuating temperatures, and variable loads remains under-explored. Assessing long-term signal stability, detection accuracy, and the impact of environmental factors such as hydraulic surges and temperature changes is crucial for evaluating the practical feasibility of MFCSe-based sensors. A robust field validation is needed to ensure that these devices will continue to operate reliably over extended periods without frequent recalibration or replacement.

Based on the problem statement above, the research questions can be formulated as below:

- a. What are the optimal surface modification strategies for a sensor to achieve enhanced accuracy and stability for environmental monitoring applications?
- b. How do the design and configuration of the sensors influence its sensitivity, stability, and robustness for pollutant detection under various simulated wastewater conditions?
- c. How effectively can an Internet of Things (IoT)-enabled platform using mathematical algorithms facilitate real-time data processing and automated alarm notifications for timely detection of abnormal water quality events?
- d. What are the long-term signal stability, pollutant detection performance, and operational reliability of the integrated MFCSe system when subjected to actual municipal wastewater treatment plant conditions?

### 1.3 RESEARCH HYPOTHESES

Based on the research questions raised, a few hypotheses have been made as below:

- a. The materials selection influences the sensitivity, response time, detection limit, electrochemical performance of the sensors.

- b. Beyond material choices, the design and configuration of the sensors impact their sensitivity, response time, detection limit, and electrochemical performance.
- c. Real-time monitoring of wastewater quality using these sensors yields accurate and reliable data and responses.
- d. Validating the reliability of the sensors necessitates testing under actual wastewater treatment conditions.

#### **1.4 RESEARCH OBJECTIVES**

Following the problem statements mentioned, the research objectives are listed as below:

- a. To construct a two-electrode microbial electrochemical sensor (MESe) using surface-modified electrodes for practical environmental monitoring applications.
- b. To evaluate a tubular-type microbial fuel cell sensor (MFCSe) with an air-cathode on its sensitivity, stability, and robustness for detecting pollutants under various wastewater conditions.
- c. To integrate an Internet of Things (IoT)-enabled system utilizing mathematical models for real-time data processing and automated alarm notification.
- d. To assess the long-term signal stability, pollutant detection performance, and operational reliability of the MFCSe system when deployed under actual municipal wastewater treatment conditions.

## **1.5 RESEARCH SCOPE**

This research focuses on developing and validating an integrated microbial electrochemical sensor (MESe) system with Internet of Things (IoT) capabilities for real-time wastewater quality monitoring. The study begins with the construction and optimization of a two-electrode MESe through surface modification strategies for 304L stainless steel electrodes, aiming to enhance microbial adhesion, electron transfer, and overall sensor accuracy and stability. Following this, the research will encompass the design and fabrication of a microbial fuel cell sensor (MFCSe), specifically a novel tubular-type configuration with an air-cathode, to improve its sensitivity, stability, and robustness for pollutant detection. Both MESe and MFCSe will undergo rigorous performance evaluation under simulated wastewater conditions, assessing key parameters like current density, COD correlation, signal linearity, and response time, with a comparative analysis to identify the optimal biosensing configuration. A critical phase involves integrating an IoT-enabled platform with the sensor system, utilizing mathematical algorithms such as Limit-of-Detection (LOD) and Rate-of-Change (RoC) for real-time data processing and automated alarm notifications for abnormal water quality events. Finally, the research will include field deployment of the integrated MFCSe system in an actual municipal wastewater treatment plant to assess its long-term signal stability, pollutant detection performance, and operational reliability under real-world conditions. This study is limited to pollutant detection in synthetic and real wastewater samples, with performance primarily assessed via electrochemical signals and sensor reliability, ultimately aiming to establish a foundation for low-cost, scalable, IoT-integrated biosensors for practical environmental monitoring in Malaysia and similar regions.

## **1.6 CONTRIBUTION TO THE BODY OF KNOWLEDGE**

This research significantly improves microbial electrochemical biosensors, helping address current challenges in environmental monitoring through new sensor designs, material science, and digital connections.

First, we constructed a two-electrode microbial electrochemical sensor (MESe) using surface-modified 304L stainless steel electrodes. This created a cost-effective and scalable platform for detecting organic pollutants based on electrical current. Our work also clarifies how specific electrode surface traits, like roughness and chemical makeup, directly affect how microbes attach and transfer electrons, which is key to making sensors accurate and stable. Second, we designed and thoroughly tested a new tubular-type microbial fuel cell sensor (MFCSe) with an air-cathode. This sensor shows better sensitivity, stability, and adaptability for pollutant detection. We also compared the performance of both MESe and MFCSe designs under controlled and real-world conditions, offering valuable insights into which sensor structures work best for different environmental situations.

Finally, a key part of this work involves integrating an Internet of Things (IoT)-enabled system with the MFCSe. This system uses mathematical algorithms (Limit-of-Detection and the Rate-of-Change) to process data in real-time and send automated alarms when water quality changes. This kind of real-time, self-operating wastewater monitoring has seen limited exploration, especially in regions like Malaysia. We also performed a long-term field deployment of the integrated MFCSe system at an actual municipal wastewater treatment plant. These real-world tests provide strong evidence of the system's operational reliability and practical value. Overall, this study links fundamental electrochemical sensor research with practical environmental monitoring technology. It sets the stage for future smart, sustainable, and decentralized pollution detection systems.

## CHAPTER II

### LITERATURE REVIEW

#### 2.1 WATER QUALITY MONITORING

##### 2.1.1 Importance of water quality monitoring

Water quality monitoring involves the systematic sampling and analysis of water to evaluate its physical, chemical, and biological characteristics, such as pH, dissolved oxygen (DO), heavy metals, nutrients, and pathogenic presence. This process ensures water is safe for drinking, suitable for agriculture or industry, and supportive of aquatic ecosystems. By employing standardized techniques, monitoring identifies contaminants and tracks changes, serving as a critical tool for pollution control. Its significance is most evident in protecting human health, where detecting pathogens like *Escherichia coli* (*E. coli*) or toxic compounds prevents diseases caused by long-term exposure. In urban areas, pollution risks are usually elevated and water quality monitoring provides essential data to pinpoint contamination sources, enabling swift actions like treatment upgrades or source isolation.

Beyond human health and ecosystems, water quality monitoring plays a crucial role in protecting wastewater treatment plant (WWTP) equipment from damage caused by harmful substances (Matheri et al. 2022). Treatment facilities rely on mechanical components, biological reactors, and chemical processes to treat wastewater, but corrosive chemicals, abrasive solids, or toxic pollutants can disrupt operations. For instance, high levels of acids or alkalis from industrial discharges can corrode tank surface, while heavy metals like cadmium may poison microbial communities at the

trickling filter essential during the biological treatment. Monitoring influent wastewater for harmful pollutants allows operators to make decision to dilute inflows or add neutralizing agents before reaching the treatment plant can prevent damage to the equipment. By having a competent wastewater monitoring system, it extends equipment lifespan, reduces maintenance costs, and prevents costly shutdowns (Forhad et al. 2024). In short, identifying harmful substances ensures treatment plants operate efficiently, maintaining their capacity to deliver clean water to the residents.

Water quality monitoring plays an essential role in regulatory compliance, ensuring that environmental laws protect both human's health and nature systems. Standards established by organizations such as the World Health Organization (WHO) has specified acceptable contaminant levels in drinking water, wastewater, and surface water (Shah et al. 2023). Therefore, water quality monitoring verifies compliance with these limits, which also directly supports the 6<sup>th</sup> Sustainable Development Goal (SDG) for ensuring the availability and sustainable management of water. With climate change affecting water availability and population growth increasing pollution loads, wastewater monitoring supplies important data to the government to take appropriate actions. This can be done by identifying emerging risks, such as novel pollutants or illegal discharges, thereby facilitating proactive governance and ensuring water resources remain sustainable. Given these demands, various conventional monitoring methods have been established.

### **2.1.2 Conventional water quality monitoring methods**

Conventional water quality monitoring methods rely on physicochemical and biological analysis to determine the presence of contaminants such as organic matter, heavy metals, and pathogens (Sangamnere et al. 2023). Despite their widespread use, these conventional techniques often require laboratory-based analysis, skilled personnel, and expensive equipment. These methods have been refined over decades and continue to serve as the benchmark in wastewater management due to their reliability and established procedures. However, their limitations in terms of cost, time, and accessibility on-site have encouraged alternative approaches, such as

bioelectrochemical system (BES)-based sensors (Altowayti et al. 2022; Shamshad et al. 2025).

One of the most widely used conventional techniques is biochemical oxygen demand (BOD) analysis, which quantifies the amount of oxygen required by microorganisms to degrade organic matter in a sample over a fixed period, typically five days (BOD<sub>5</sub>). This method involves incubating a wastewater sample under controlled conditions and measuring oxygen depletion using dissolved oxygen probes. While BOD<sub>5</sub> provides an accurate indicator of organic pollution, its long incubation period makes it impractical for real-time monitoring (Verma et al. 2021). Complementary to BOD, chemical oxygen demand (COD) uses chemical oxidants to estimate organic content more quickly, usually within few hours. COD requires hazardous reagents and specialized equipment, which poses safety and disposal challenges (Lambertz et al. 2024; Singh et al. 2021). Additionally, both methods require controlled laboratory conditions and trained researchers to perform such analyses, making it less suitable for immediate response scenarios, such as detecting a sudden pollution event or illegal discharges.

Another key aspect of conventional wastewater monitoring is detecting contaminants like heavy metals (lead, cadmium, mercury, and chromium) and nutrients (ammonia, phosphorus, potassium, and sulfur) using techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and ion chromatography (IC). These methods are excellent in identifying trace pollutants at very low concentrations that can harm ecosystems or health if not addressed. Heavy metals often come from industrial waste, while nutrients stem from sewage or agricultural runoff, causing issues like algal blooms (J. Wang et al. 2022). Their high sensitivity and precision are essential for meeting environmental standards. For instance, AAS employs a flame or furnace to vaporize a wastewater sample, subsequently quantifying the absorption of light by specific metals at their own distinct wavelengths, thereby providing a unique spectral profile for each element (Omeje et al. 2021; Perelsonia et al. 2021). On the other hand, ICP-MS uses an inductively coupled plasma to ionize samples and convert them to ions, which are then detected by mass (Balaram et al. 2023). Ion chromatography separates nutrient ions in a column and

measuring their levels with a detector (Liu et al. 2021). These lab-based instruments deliver reliable results but they demand meticulous sample collection, transport, and pretreatment, which can delay outcomes and risk errors if mishandled. These drawbacks highlight the need for alternatives like bioelectrochemical system (BES)-based sensors, which could offer a rapid response.

Next, biological assays are vital in conventional wastewater analysis, particularly for evaluating microbial contamination and toxicity, which directly affect public health and ecosystems (Luan et al. 2020). These methods assess the presence of pathogens or the harmful effects of pollutants by studying living organisms' responses. One key approach is detecting the presence of fecal coliform bacteria such as *Escherichia coli* (*E. Coli*) that could spread diseases like cholera or gastroenteritis. Techniques like membrane filtration involve passing a wastewater sample through a fine filter to trap bacteria, which are then grown on a nutrient-rich plate to count the colonies. The most probable number (MPN) method, alternatively, dilutes samples in tubes to estimate bacterial numbers based on growth patterns. These tests ensure wastewater is safe for release, which can help to protect communities from health risks (Yang et al. 2016). However, pathogen detection methods are highly time consuming, often taking days to yield results due to the incubation periods of pathogens. Moreover, these biological methods demand expertise to interpret results accurately, as subtle changes in organism behavior or bacterial counts can be complex to analyze, which is not practical for rapid detection of harmful pathogens in wastewater.

In short, the aforementioned conventional wastewater monitoring approaches including oxygen demand assessments, sophisticated analytical instruments, and pathogenic-based tests provide reliable and precise results in determining the water quality. However, the testing process must be performed offsite and can take several hours or days to complete, which is not feasible to identify sudden pollution issues, like unexpected spills or illegal discharges. Additionally, these methods depend heavily on high-end laboratories, skilled technicians, and costly equipment as well, which impose huge financial burden. From a critical standpoint, these conventional techniques seem to focus significantly on the precision of laboratory results at the expense of flexibility and quickness. This makes them less suited to the demands of wastewater monitoring,

where management is seeking rapid, on-site and low-cost solutions. This has driven interest in novel technologies, particularly bioelectrochemical system (BES)-based sensors, which hold potential to overcome these constraints by enabling faster, more accessible, and possibly self-powered monitoring solutions, aligning better with the needs of sustainable and responsive wastewater systems.

### **2.1.3 Background of key pollutants in wastewater monitoring**

Wastewater streams often contain a wide range of organic compounds and toxic substances originating from domestic, industrial, and agricultural activities. These pollutants differ significantly in their biodegradability, toxicity, and impact on biological treatment processes (Sá et al. 2022; J. Zhang et al. 2021). Understanding the characteristics of representative pollutants is essential for evaluating water quality monitoring technologies especially for BES-based sensors, which rely on microbial metabolic activity for signal generation (Shanbhag et al. 2023). In this study, several model pollutants were selected to represent different categories of wastewater contamination, including biodegradable organics, complex industrial effluents, and toxic inhibitory compounds.

#### **a. Readily biodegradable organic substrates**

Sodium acetate is a low-molecular-weight organic salt commonly present in domestic wastewater and widely used as a supplementary carbon source in biological wastewater treatment processes (Idris et al. 2023). Due to its simple molecular structure, acetate is readily metabolized by a wide range of microorganisms and is frequently used as a model substrate in biochemical oxygen demand (BOD), chemical oxygen demand (COD), and bioelectrochemical studies (Qiu et al. 2022). In BES-based systems, acetate serves as a preferred electron donor for electroactive bacteria, enabling stable biofilm formation and predictable electron transfer behavior (Mollaei et al. 2021). As such, sodium acetate is often employed as a reference compound to evaluate baseline sensor performance, microbial activity, and system sensitivity under non-toxic conditions.

Ethanol is another easily biodegradable organic compound commonly found in wastewater from food processing, beverage production, pharmaceutical manufacturing, and chemical industries (Rodriguez-Caballero et al. 2012). Ethanol is rapidly oxidised by microorganisms, leading to a quick increase in metabolic activity and oxygen or electron demand. In wastewater monitoring contexts, ethanol represents short-chain alcohol contamination and sudden increases in readily biodegradable organic loading. Its inclusion as a test pollutant allows assessment of sensor responsiveness to rapid changes in substrate availability and organic strength, which are common during industrial discharge events or process upsets (Mukimin et al. 2023).

**b. Complex industrial and agro-industrial wastewater**

Palm Oil Mill Effluent (POME) is a highly concentrated agro-industrial wastewater generated during palm oil extraction processes. It is characterized by extremely high COD and BOD values, elevated suspended solids, oil and grease content, and a complex mixture of organic compounds (Kacaribu et al. 2025). In Malaysia, POME is one of the most significant industrial wastewater streams and poses serious environmental risks if improperly treated. The complex and variable composition of POME presents challenges for conventional monitoring techniques and biological treatment systems (Kadier et al. 2022). Including POME in wastewater monitoring studies is important for evaluating sensor robustness, signal stability, and performance under realistic, high-strength wastewater conditions that reflect local industrial practices (Yeo et al. 2023).

Paint thinner refers to a mixture of organic solvents commonly used in coating, printing, and manufacturing industries. These solvents may include aromatic hydrocarbons, ketones, and alcohols, many of which are toxic or inhibitory to microbial communities (Ghosh et al. 2025). Wastewater contaminated with thinner residues can disrupt biological treatment processes by suppressing microbial activity and damaging cell membranes (Güven et al. 2017). From a monitoring perspective, thinner represents solvent-based industrial pollution that may occur through illegal discharge or accidental spills. Its presence in wastewater highlights the need for early detection systems capable of identifying inhibitory compounds before they cause irreversible damage to treatment performance (Nair K et al. 2021).

### c. Toxic organic pollutants and inhibitors

4-Nitrophenol is a phenolic compound widely used as an intermediate in the production of pesticides, dyes, pharmaceuticals, and explosives (Cardoso Juarez et al. 2024). It is recognised as a priority pollutant due to its toxicity and inhibitory effects on microbial metabolism. Even at low concentrations, 4-nitrophenol can disrupt enzymatic activity and electron transport processes in microorganisms (Sarkar et al. 2022). In wastewater treatment systems, its presence may lead to reduced biodegradation efficiency and system instability (Z. Zhang et al. 2021, 2022). As a model toxicant, 4-nitrophenol is frequently used in biosensor research to evaluate the ability of sensing platforms to detect acute toxicity and inhibitory shock events (Vignesh et al. 2026).

Methyl orange is a synthetic azo dye commonly discharged from textile, paper, and laboratory operations. Azo dyes are known for their complex aromatic structures (El-Sonbati et al. 2015), which make them resistant to biodegradation and potentially toxic to aquatic organisms (Huang et al. 2018). Methyl orange can interfere with microbial respiration and electron transfer (Kataya et al. 2025; Khan et al. 2022), particularly under aerobic and bioelectrochemical conditions (Chen et al. 2024). In wastewater monitoring studies, dyes such as methyl orange are often used to represent recalcitrant organic pollutants and to assess the sensitivity of sensors toward compounds that do not readily contribute to microbial energy generation but still exert inhibitory effects (Romain Njimou et al. 2024; Wu et al. 2024).

Malathion 57 is an organophosphate pesticide extensively used in agricultural pest control. Runoff from agricultural land, improper storage, and accidental spills can introduce malathion into surface water and wastewater systems. Organophosphate compounds are known to inhibit key enzymes involved in microbial metabolism and pose risks to both environmental and human health (Tanveer et al. 2024). In biological treatment systems, malathion can cause partial or complete inhibition of microbial activity (Suhane et al. 2026), leading to reduced treatment efficiency. Its inclusion in wastewater monitoring research enables evaluation of sensor capability to detect pesticide-related toxic shocks, which are particularly relevant in regions with intensive agricultural activity.

### 2.1.4 Regulatory standards for wastewater discharge in Malaysia

In Malaysia, the Department of Environment (DOE) enforces regulatory limits on wastewater discharge to protect inland waters and public health under the Environmental Quality (Industrial Effluent) Regulations 2009, which are issued pursuant to the Environmental Quality Act 1974. These regulations prescribe different concentration limits depending on the sensitivity of receiving environments, commonly classified as Standard A and Standard B. Standard A applies when effluent is discharged into sensitive water bodies such as upstream of water supply intake points or environmentally critical areas, while Standard B applies to less sensitive receiving waters downstream of abstraction points. The regulated parameters include physicochemical indicators such as temperature, pH value, BOD, COD, and suspended solids. Similarly, prioritized pollutants such as heavy metals and phenolic compounds were also monitored heavily due to the impacts of such pollutants towards the water body. Compliance with these standards (Table 2.1) necessitates adequate wastewater treatment and continuous monitoring, underscoring the importance of reliable real-time water quality surveillance technologies like BES sensors in supporting environmental protection and regulatory adherence.

Table 2.1 Acceptable conditions for discharge of industrial effluent or mixed effluent of standards A and B ("Environment Quality (Industrial Effluent) Regulations 2009.").

| Parameter                 | Unit               | Standard A | Standard B |
|---------------------------|--------------------|------------|------------|
| Temperature               | °C                 | 40         | 40         |
| pH value                  | -                  | 6.0 – 9.0  | 5.5 – 9.0  |
| BOD <sub>5</sub> at 20 °C | mg L <sup>-1</sup> | 20         | 50         |
| COD                       | mg L <sup>-1</sup> | 80         | 200        |
| Suspended solids          | mg L <sup>-1</sup> | 50         | 100        |
| Mercury                   | mg L <sup>-1</sup> | 0.005      | 0.05       |
| Cadmium                   | mg L <sup>-1</sup> | 0.01       | 0.02       |
| Chromium hexavalent       | mg L <sup>-1</sup> | 0.05       | 0.05       |
| Arsenic                   | mg L <sup>-1</sup> | 0.05       | 0.10       |
| Cyanide                   | mg L <sup>-1</sup> | 0.05       | 0.10       |
| Lead                      | mg L <sup>-1</sup> | 0.10       | 0.5        |
| Chromium trivalent        | mg L <sup>-1</sup> | 0.20       | 1.0        |
| Copper                    | mg L <sup>-1</sup> | 0.20       | 1.0        |
| Manganese                 | mg L <sup>-1</sup> | 0.20       | 1.0        |

... to be continued

... continuation

|                     |                    |       |      |
|---------------------|--------------------|-------|------|
| Nickel              | mg L <sup>-1</sup> | 0.20  | 1.0  |
| Tin                 | mg L <sup>-1</sup> | 0.20  | 1.0  |
| Zinc                | mg L <sup>-1</sup> | 1.0   | 1.0  |
| Boron               | mg L <sup>-1</sup> | 1.0   | 4.0  |
| Iron                | mg L <sup>-1</sup> | 1.0   | 5.0  |
| Phenol              | mg L <sup>-1</sup> | 0.001 | 1.0  |
| Free Chlorine       | mg L <sup>-1</sup> | 1.0   | 2.0  |
| Sulphide            | mg L <sup>-1</sup> | 0.50  | 0.50 |
| Oil and grease      | mg L <sup>-1</sup> | ND    | 10   |
| Silver              | mg L <sup>-1</sup> | 0.1   | 1.0  |
| Aluminum            | mg L <sup>-1</sup> | 10    | 15   |
| Selenium            | mg L <sup>-1</sup> | 0.02  | 0.5  |
| Barium              | mg L <sup>-1</sup> | 1.0   | 2.0  |
| Fluoride            | mg L <sup>-1</sup> | 2.0   | 5.0  |
| Formaldehyde        | mg L <sup>-1</sup> | 1.0   | 2.0  |
| Ammoniacal nitrogen | mg L <sup>-1</sup> | 10    | 20.0 |
| Color               | ADMI*              | 100   | 200  |

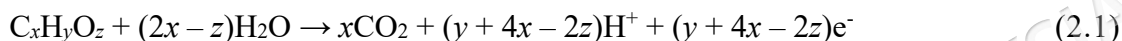
\*ADMI – American Dye Manufacturers Institute

## 2.2 BIOELECTROCHEMICAL SYSTEM (BES)

Bioelectrochemical systems (BES) are advanced technologies that harness microbial metabolic processes to generate electrical currents through electrochemical reactions (Jadhav et al. 2025). These systems employ electroactive microorganisms to catalyze the oxidation of organic and inorganic substrates, producing electrons that drive electrical outputs. By integrating electrochemical and biological principles, BES offer various applications including electricity generation (Choudhary et al. 2024), biofuel and biogas production (Sharma et al. 2023), wastewater treatment (Varjani. 2022), and biosensing (Shanbhag et al. 2023). In terms of wastewater monitoring, their unique ability to convert chemical energy into electrical signals distinguishes them from traditional monitoring approaches, providing opportunities for innovative solutions. The effectiveness of BES relies on their underlying mechanisms and electron transfer processes, which are critical to their functionality. The following subsections examine the operational principles of BES and the electron transfer mechanisms enabling their performance.

### 2.2.1 BES mechanism

Bioelectrochemical systems (BES) rely on the interaction of microbial metabolism and electrochemical processes, where electrogenic microorganisms convert chemical energy from substrates into electrical energy (Figure 2.1). In a typical dual-chamber BES, an anode and cathode are separated by an ion exchange membrane. The anode chamber is typically maintained under anaerobic conditions and hosts electroactive bacteria, which undergoes bioelectrocatalytic oxidation of organic substrates with a general equation as below:



For instance, the anodic oxidation reactions for glucose (Equation 2.2) and acetate (Equation 2.3) are:



This process releases electrons to the anode and protons into the electrolyte. The anode is often comprised of highly conductive materials to serve as electron acceptors, which then flow through an external circuit to generate current. On the other hand, protons migrate through the cation exchange membrane (CEM) or proton exchange membrane (PEM) to the cathode chamber. The membrane's selective permeability is critical as it allows proton transfer while preventing oxygen crossover into the anode, which maintains the anaerobic conditions and ensures the ionic balance between chambers.

In the cathode chamber, reduction reactions complete the electrochemical process. Electrons from the external circuit combine with oxygen and protons generated from the anodic chamber to form water through the reduction equation below:



This cathodic reaction is essential as it completes the electrochemical circuit, enabling sustained electron flow from the anode to the cathode. The cathode material must exhibit high electrical conductivity and resistance to corrosion to ensure reliable performance. Moreover, the efficiency of the reduction process depends significantly on the presence and accessibility of oxygen or alternative terminal electron acceptors. Hence, the oxygen reduction reaction (ORR) at the cathode chamber is often considered as the bottleneck of BES. Understanding the electron transfer mechanism driving these anodic and cathodic processes is crucial for optimizing BES performance, as discussed in the following subsection.

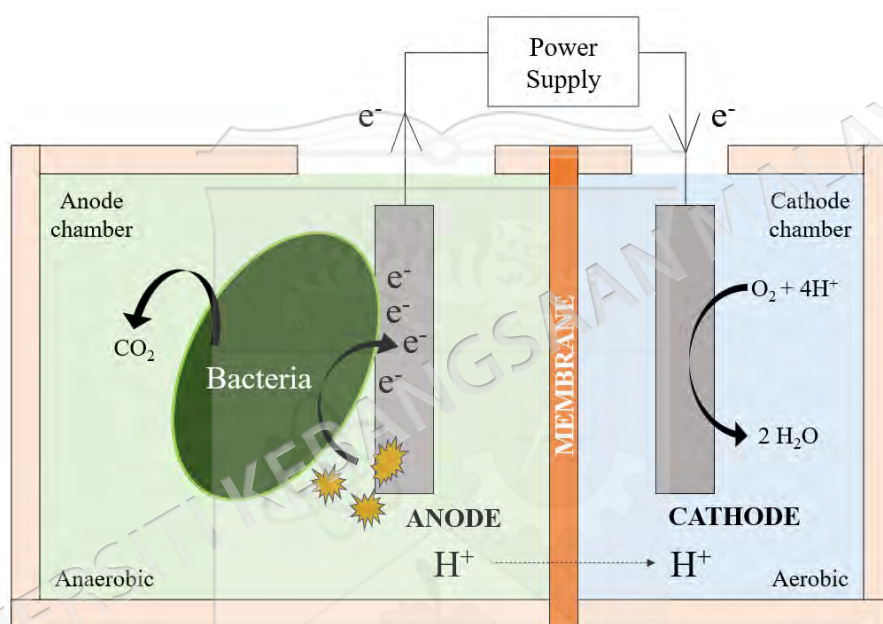


Figure 2.1 Mechanism of Bioelectrochemical systems (BES).

### 2.2.2 Electron transfer mechanisms

Understanding how electrogenic microorganisms produce electricity is crucial for assessing their role in bioelectrochemical systems (BES). A key aspect of this process is elucidating the mechanisms by which electrons are transferred between the microbes and electrodes. Electron transfer can occur through two primary mechanisms: direct and indirect electron transfer.

**a. Direct electron transfer (DET)**

Direct electron transfer (DET) is a fundamental mechanism in BES, where electrons are transferred between the electrogenic microorganisms and the electrodes without relying on external mediators (Logan et al. 2019). This mechanism is highly efficient and primarily facilitated by electrochemically active microorganisms such as *Geobacter* sp. (Guo et al. 2023) and *Shewanella* sp. (Zang et al. 2023) in BESs (Yang et al. 2012). In order for the DET to occur, the mechanism requires direct physical contact between the electron-producing microbes and the electrode surface, which allows the electron transfer through membrane-bound proteins or conductive bacterial structures.

One of the key components in DET is outer membrane cytochromes, which are responsible for transferring electrons from intracellular microbial metabolic reactions to the extracellular environment by forming a bridge between the cells and electrodes for the electron flow (Gralnick et al. 2023; Guo et al. 2022). For instance, Zhang et al. reported that visible light illumination boosted the extracellular electron transfer in *Geobacter sulfurreducens* more than 8-fold compared to dark conditions by altering the outer membrane c-type cytochromes (OM c-cyts) to an excited state (B. Zhang et al. 2021). In addition to cytochromes, certain bacteria produce electrically conductive appendages known as bacterial nanowires or pili. Notably, *Shewanella Oneidensis* MR-1 generates nanowires that extend from the outer membrane to the electrode surface allowing long-range electron transfer (Munoz-Cupa et al. 2024), which facilitate electron transfer across distances greater than direct membrane-electrode contact (Figure 2.2).

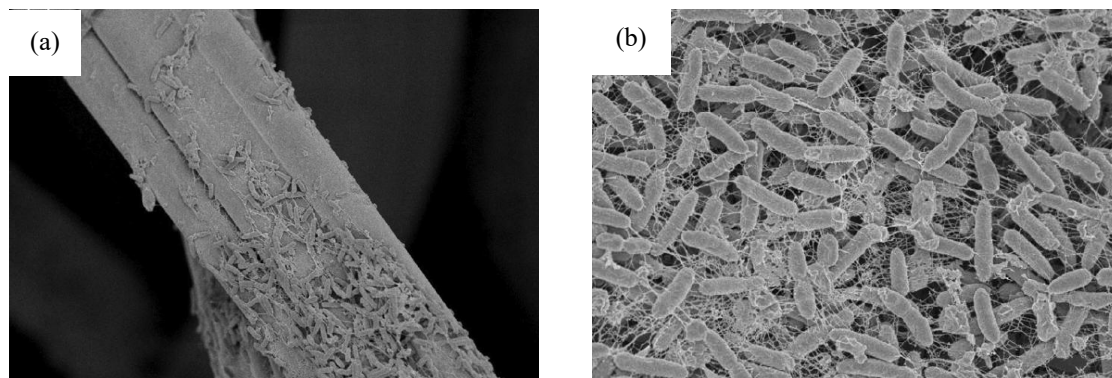


Figure 2.2 Scanning electron microscope (SEM) images of *Shewanella Oneidensis* MR-1 biofilm (a) developed on anode carbon felt fibers and (b) with nanowires.

Biofilm formation is another significant factor in influencing the DET. In numerous BES applications, electrogenic microorganisms proliferate on the electrode surface and develop dense biofilms. These biofilms form a highly conductive microbial network that boosts the electron transfer efficiency. Hence, the development of these biofilms is highly driven by the electrode material and architecture (Astorga et al. 2020; Ratheesh et al. 2021).

Generally, DET provides high electron transfer efficiency since this mechanism does not rely on external mediators, which leads to a steady power output in BES (Thengumthottathil et al. 2024). This enhances the voltage production and degradation of organic pollutants by allowing the microbes to use electrodes as electron acceptors. Despite its advantages, DET is often limited by the slow biofilm development and the requirement for suitable electrodes to maximize bacterial growth (Gong et al. 2020; Jia et al. 2021). Based on Kim et al., DET efficiency can be elevated by selecting an electrode material that satisfies the biocompatibility, has excellent electrical conductivity, and most importantly possesses a large surface area for bacteria adhesion (Kim et al. 2024).

#### **b. Indirect electron transfer (IET)**

Indirect electron transfer (IET) is another vital mechanism in BES, which is mediated by redox-active mediators secreted by electroactive microorganisms to relay electrons from the cells to the electrodes (Verma et al. 2023). In contrast to DET, IET does not

require microbial adhesion to the electrode surface, which allows electron transfer over greater distances. Redox mediators such as flavins (F. Zhang et al. 2022), quinones (Franza et al. 2022) and phenazines (Chen et al. 2013) are typical electron shuttles responsible for IET. These compounds transfer electrons by channeling the electrons generated from microbial oxidation process to the electrode. For instance, *Pseudomonas aeruginosa* produces phenazines that enhance electron shuttling as well as supporting cellular redox balancing (Schiessl et al. 2019). Next, synthetic mediators such as neutral red or methylene blue can further improve efficiency of BESs. However, these artificial redox mediators are commonly toxic to the environment, which require extensive downstream removal techniques (Gemünde et al. 2022).

In IET, microbial consortia demonstrate greater versatility compared to DET because non-electroactive bacteria can contribute in electron transport by serving as redox mediators, which further promotes symbiosis among the microbial community. Notably, the redox mediators produced by one species can also facilitate the electron transfer of other species with the electrodes (Sun et al. 2020). Therefore, it was previously reported that the power production of BES with mixed cultures was significantly higher than that of single culture BES (S. Wang et al. 2021). However, IET can be disrupted by the biosurfactants produced on the microbial cell surface, particularly during biodegradation of organic compounds in wastewater treatment (Debnath et al. 2023; Karlapudi et al. 2018).

IET offers an alternative pathway for electron transfer in BESs, which increases the rate of electron transfer in certain systems where DET is slow or inefficient. Despite its adaptability and flexibility, systems with IET generally exhibit lower electricity production compared to DET due to environmental losses and the potential toxicity of synthetic mediators. Mediator degradation over time can also compromise BES stability (Bankole et al. 2021; Kwak et al. 2019), necessitating continuous mediator replenishment. Another method to ameliorate the rate of electron transfer is by incorporating an electron channel within the cell. For instance, the introduction of electron channel MtrABC into *E. coli* allows the cells to transfer electrons *via* direct contact with the electrodes (Liang et al. 2024). In short, advances in mediator design, electrolyte composition, and highly biocompatible electrode materials are essential to

address these challenges, thereby improving IET applicability in BESs. These electron transfer processes support both energy production and the development of sensitive detection systems in BES, as discussed in the upcoming section on the types of BES-based sensors.

### **2.2.3 Bioelectrochemical system (BES)-based sensor**

Bioelectrochemical systems (BESs) have evolved into sensor technologies that utilize electroactive biofilms to enhance electron transfer on electrodes for monitoring environmental parameters. The electrical signals generated by these sensors can be correlated with specific factors, such as pollutant concentration. In wastewater monitoring, the electrical output generated by BES-based sensors is closely linked to the concentration of biodegradable organic matter, commonly represented by BOD (Lin et al. 2022). As BOD increases, electroactive microorganisms oxidize a greater number of organic substrates, resulting in enhanced metabolic activity and increased electron transfer to the anode. This process leads to a higher current or voltage signal, providing an indirect yet continuous indication of organic pollution levels. Conversely, a reduction in biodegradable organics or the presence of inhibitory compounds can suppress microbial activity and cause a measurable decline in electrical output. This intrinsic relationship between microbial metabolism and electrochemical response enables BES sensors to function as real-time indicators of BOD variation, offering advantages over conventional laboratory-based BOD measurements that are time-consuming and discontinuous. Two prominent types of BES-based sensors are microbial electrochemical sensors (MESe) and microbial fuel cell sensors (MFCSe), which will be discussed in detail in the following subsections.

### **2.2.4 Existing MESe with stainless steel electrodes**

In the domain of MESe, the choice of electrode material is critical for system performance and scalability. As outlined in Table 2.2, a range of studies has explored the use of stainless steel electrodes due to their cost-effectiveness, durability, and corrosion resistance. These characteristics make stainless steel a practical alternative to more expensive carbon-based materials for long-term applications. Research has

demonstrated that stainless steel electrodes can successfully support electrogenic biofilm formation and provide a stable platform for a variety of biosensing applications, including the monitoring of organic matter and pollutants. The tabulated studies provide clear evidence of stainless steel's efficacy as a low-cost and robust electrode material, validating its use as a viable option for developing sustainable and efficient MESes for environmental monitoring.

Table 2.2 A summary of stainless steel anodes and their modifications in MESes.

| Anode         | Cathode                      | Modification of anode                                                               | Improvement                                                                                     | Output                  | References            |
|---------------|------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------|-----------------------|
| 316L SS felt  | SS mesh                      | Flame oxidised SS felt at approximately 1200°C using a Bunsen burner for 60 seconds | Enhanced bioelectricity generation, improved biofilm formation                                  | 19.20 A m <sup>-2</sup> | (Guo et al. 2014)     |
| SS fibre felt | Carbon felt                  | Coating of graphene, carbon nanotube and activated carbon on SS fibre felt          | Enlarged surface area, enhanced bioelectricity generation                                       | 6.10 A m <sup>-2</sup>  | (Hou et al. 2014)     |
| 316L SS felt  | SS mesh                      | Heat treatment of SS felt in a muffle furnace at 600°C for 5 minutes                | Enhanced bioelectricity generation, improved biofilm formation                                  | 15.00 A m <sup>-2</sup> | (Guo et al. 2015)     |
| SS mesh       |                              |                                                                                     | Enlarged three-dimensional surfaces, minimized ohmic losses, enhanced bioelectricity generation | -                       | (Erbay et al. 2015)   |
|               | Platinum-loaded carbon paper | Multiwall carbon nanotubes were grown on SS mesh                                    |                                                                                                 |                         |                       |
| SS coupons    | Platinum wires               | Flame oxidised SS coupons with a handheld butane torch                              | Enhanced bioelectricity generation                                                              | 27.22 A m <sup>-2</sup> | (Ledezma et al. 2015) |
| SS mesh       | SS mesh                      | Exfoliated graphite-multiwall carbon nanotube pasted on SS mesh                     | Minimized start-up time, reduced activation resistance                                          | -                       | (Song et al. 2015)    |

... to be continued

... continuation

|          |                          |                                                                  |                                                                                                                |                     |
|----------|--------------------------|------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------|
| SS mesh  |                          |                                                                  | Enhanced bioelectricity generation, improved mechanical and electrical properties, improved microbial adhesion | (Zheng et al. 2015) |
|          | Graphite plate           | Carbon black coating on SS mesh by dipping/drying process        | 15.30 A m <sup>-2</sup>                                                                                        |                     |
| SS mesh  |                          |                                                                  | Enhanced bioelectricity generation, improved corrosion resistance                                              | (Peng et al. 2016)  |
|          | Graphite plate           | Carbon black coating on SS mesh by dipping/drying process        | 19.10 A m <sup>-2</sup>                                                                                        |                     |
| SS plate |                          |                                                                  | Enhanced bioelectricity generation, improved corrosion resistance                                              | (Pu et al. 2018)    |
|          | Titanium plate           | In situ electrochemical deposition of polypyrrole onto SS plate  | 1.37 A m <sup>-2</sup>                                                                                         |                     |
| SS foil  | Carbon cloth air cathode | Carbon nanofibers-polydimethylsiloxane paste was cast on SS foil | -                                                                                                              | (Saadi et al. 2020) |
|          |                          |                                                                  | Enhanced bioelectricity generation, improved corrosion resistance, improved biocompatibility                   |                     |

The advantages of stainless steel as a durable, cost-effective, and corrosion-resistant electrode material for MESes can be established and explored in this research. While this body of work provides a strong foundation for developing robust biosensors, a key limitation of MESes is their reliance on an external power supply to drive the cathodic ORR reaction, which is still the major bottleneck in microbial electrochemistry (Rezaei et al. 2023). These challenges have motivated a shift in research towards self-powered systems, which are addressed by the development of MFCSe technology. This has led to the exploration of alternative system designs, as will be discussed in the following section on existing MFCSe with modified air-cathodes that are constantly exposed to the atmospheric oxygen.

### 2.2.5 Existing MFCSe with modified air-cathodes

A crucial innovation addressing the limitations of MESEs has been the development of microbial fuel cell sensors (MFCSe), particularly those incorporating modified air-cathodes. Unlike MESEs, which require an external power supply and are limited by the cathodic reaction bottleneck, MFCSe are self-powered systems that harness atmospheric oxygen as the terminal electron acceptor. This design eliminates the need for external energy and provides a stable and continuous power source, making the sensors suitable for long-term, autonomous field monitoring. The efficiency of these systems is heavily dependent on the performance of the air-cathode, which has led to extensive research into modifying cathode materials with various catalysts to enhance the ORR and bioelectricity generation. The following table summarizes key studies and the diverse modifications applied to air-cathodes in existing MFCSe systems (Table 2.3).

Table 2.3 A summary of air-cathodes and their modifications in MFCSe.

| Air-cathode                                                          | Anode                                                                | Modification of air-cathode                                                                                | Improvement                                                                                         | Output                         | References                    |
|----------------------------------------------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------|-------------------------------|
| Carbon cloth PVA-MEA air-cathode                                     | Graphite felt                                                        | Carbon cloth pressed on denatured 10 wt% polyvinyl alcohol (PVA) forming membrane-electrode assembly (MEA) | Improved ORR reaction, enhanced bioelectricity generation                                           | 59 – 143 mW m <sup>-2</sup>    | (Liu et al. 2017)             |
| Carbon fiber felt, stainless steel mesh, graphite rod, foamed nickel | Carbon fiber felt, stainless steel mesh, graphite rod, foamed nickel | -                                                                                                          | Carbon fiber felt and foamed nickel suitable for wastewater treatment and bioelectricity generation | 2.30 – 5.11 mW m <sup>-2</sup> | (Wang et al. 2017)            |
| Carbon cloth air-cathode                                             | Carbon felt                                                          | PbO <sub>2</sub> layer of 0.5 mg cm <sup>-2</sup>                                                          | Enhanced bioelectricity generation, improved wastewater treatment                                   | 24 mA m <sup>-2</sup>          | (Estrada-Arriaga et al. 2018) |
| Activated carbon air-cathode                                         | Carbon fiber brush                                                   | Constant supply of half-wave rectified alternating fields                                                  | Improved recovery of bioelectricity generation up to                                                | -                              | (Zhou et al. 2018)            |

... to be continued

... continuation

|                                         |                      |                                                                          |                                                                 |                          |                         |
|-----------------------------------------|----------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------|-------------------------|
|                                         |                      |                                                                          | 50%, reduced biofouling                                         |                          |                         |
| Carbon cloth air-cathode                | Carbon cloth         | Addition of cobalt and zinc oxide nanoparticles into the catalysts layer | Enhanced bioelectricity generation, reduced biofouling          | 773 mW m <sup>-2</sup>   | (Yang et al. 2019)      |
| Carbon cloth air-cathode                | Graphite fiber brush | Wet proofed 30% and coated with four layers of 60% PTFE solution         | Improved dye decolorization, enhanced bioelectricity generation | 23.50 mW m <sup>-2</sup> | (Dai et al. 2020)       |
| Steel mesh                              | Graphite fiber brush | N-doping of graphene aerogel                                             | Improved ORR performance, enhanced bioelectricity generation    | 1593 mW m <sup>-2</sup>  | (G. Wang et al. 2021)   |
| Monolithic free standing air-cathode    | Carbon fiber brush   | Tubular air-cathodes tailored with <i>Chlorella pyrenoidosa</i>          | Enhanced bioelectricity generation                              | 106.3 W m <sup>-2</sup>  | (Jun Li et al. 2022)    |
| Floating carbon fiber cloth air-cathode | Carbon fiber cloth   | Triple-phase interphase (TPI) with multiple hydrophobic PTFE layers      | Enhanced bioelectricity generation                              | 48.88 mW m <sup>-2</sup> | (Mohd Noor et al. 2024) |

## 2.3 BES ANODE AND CATHODE MATERIALS

In BESs, the selection of anode and cathode materials significantly impacts the system's efficiency because electrode materials directly influence the microbial adhesion, electron transfer, electricity production, and half-cell redox reactions. This section discusses the selection requirements of anode and cathode materials.

### 2.3.1 Anode materials

The anode plays a vital role in BES by serving as the interface where electroactive microorganisms transfer electrons. The performance of BES largely depends on the anode material, which affects the development of biofilm and oxidation of organic matter. Selecting an appropriate anode material requires consideration of several key criteria, including biocompatibility, electrical conductivity, corrosion resistance, and surface area.

### **a. Biocompatibility**

As mentioned in Subsection 2.5.1, The effectiveness of bioelectrochemical systems (BESs) heavily depends on the choice of electrode materials, which should be selected based on their compatibility with the intended bioelectrochemical functions. For a system to operate as a true BES, it is essential that electrogenic microorganisms adhere to the anode surface and develop into electroactive biofilms, which play a critical role in generating power. As such, ensuring that the electrode material is biocompatible becomes a fundamental consideration in anode design for BES applications. Recently, Fang et al. electrochemically modified biocompatible graphene/polyaniline (GO/PANI) on carbon paper (CP) and the resultant GO/PANI@CP electrode significantly enhanced the performance of electrogenic microbes at the anode for bioelectricity generation and wastewater treatment, producing a power density 54 times higher compared to the blank CP electrode (Fang et al. 2024). Another method to ameliorate biocompatibility is by using an anode based on natural biomass, such as mango wood-derived carbon (Li et al. 2020), maple wood biochar (Md Khudzari et al. 2019), chestnut shell-derived carbon (Chen et al. 2018), and coffee waste-derived porous carbon (Hung et al. 2019). Apart from biocompatibility, they also offer a sustainable alternative to synthetic or metal-based electrodes. In short, the literature indicates that electron-producing microbes proliferate more effectively and healthily on biocompatible anode materials. Selecting a biocompatible electrode material in BES-based sensors allows a quicker biofilm development, which ultimately reduces the startup period of the sensors.

### **b. Electrical conductivity**

Once electroactive microorganisms successfully adhere to the surface, they transition to a stable attachment phase, leading to the development of microcolonies and the eventual formation of a mature biofilm. (Greenman et al. 2021; Greenman et al. 2022). The biofilm acts as an intricate microbial community, where cells are encapsulated within extracellular polymeric substances (EPS) that support structural stability and enable extracellular electron transfer (EET) (Seviour et al. 2019). Therefore, the anode's electrical conductivity plays a critical role in determining the efficiency of the extracellular electron transfer (EET) process. Materials with high conductivity enable swift electron transport from the biofilm generated during microbial substrate oxidation

allowing electrons to move through the anode and into the external circuit with minimal resistance. (Jiang et al. 2019; Zhang et al. 2019). Consequently, the increased electron flow from the anode enhanced the cathodic oxygen reduction reaction (ORR), leading to improved overall power generation in the bioelectrochemical system (BES). In other words, the anode's electrical conductivity not only influences the efficiency of the EET process but also significantly affects the system's overall performance.

### c. Corrosion resistance

Beyond conventional considerations such as electrical conductivity and biocompatibility, anode materials used in bioelectrochemical systems (BESs) must also demonstrate strong resistance to corrosion. This is critical because electroactive microbes involved in extracellular electron transfer (EET) can trigger microbiologically influenced corrosion (MIC), leading to the structural degradation of electrode materials (Zhou et al. 2022). Since the anode serves as the primary site for microbial colonization, biofilm formation, and EET, any material breakdown due to MIC can result in elevated internal resistance and a subsequent drop in system output. Additionally, corrosion byproducts may interfere with microbial metabolic activity or disrupt the surrounding electrolyte conditions, thereby hindering electron transport and reducing energy production (Li et al. 2017). For example, copper anodes subjected to MIC yielded substantially lower power output ( $2 \text{ mW m}^{-2}$ ) compared to stainless steel ( $12 \text{ mW m}^{-2}$ ) and carbon cloth ( $880 \text{ mW m}^{-2}$ ), largely due to the toxic effects of leached copper ions on microbial growth (Zhu et al. 2014). Moreover, BES anodes must resist corrosion not only from microbial activity but also from prolonged exposure to aqueous and ionic environments. Zhu et al. observed surface defects such as cracking and blistering on graphite anodes after exposure to electrolytes like  $\text{Na}_2\text{SO}_4$ ,  $\text{NaNO}_3$ , and  $\text{NaCl}$  for just 60 minutes. (Zhu et al. 2019). More recently, Ma et al. reported that graphitic carbon nitride ( $\text{g-C}_3\text{N}_4$ ) exhibits excellent long-term and spatially extended anti-corrosive behavior, making it highly promising for use as anode material, both in its pure form and as part of composite structures (Yongning Ma et al. 2022).

### 2.3.2 Cathode materials

Similar to the anode, cathode is also a crucial component in bioelectrochemical systems (BES), responsible for receiving electrons from the external circuit and facilitating the oxygen reduction reaction (ORR). In fact, the cathodic ORR has been a major bottleneck in BESs

#### a. Catalytic performance

In contrast to the criteria used for selecting MFC anode materials which prioritize the promotion of electroactive biofilm development and efficient extracellular electron transfer (EET), the design of MFC cathode materials primarily centers on catalytic activity and reaction selectivity. An effective cathode must exhibit high catalytic efficiency to drive the oxygen reduction reaction (ORR), a process widely recognized as the rate-limiting step in microbial fuel cells (Song et al. 2024; Zhuang et al. 2020). One of the key strategies for evaluating ORR catalyst performance involves analyzing the binding energies of reaction intermediates such as  $\text{OH}^*$  and  $\text{OOH}^*$ . These energies are often visualized in a volcano plot, which helps researchers determine optimal materials and guide catalyst development (Stratton et al. 2023). Platinum and its alloys typically appear near the top of the volcano plot due to their ideal binding characteristics and minimal overpotential during ORR (T. Wang et al. 2021). As a result, extensive efforts have been directed toward developing platinum-based catalysts. However, the high cost of platinum has driven the exploration of more affordable alternatives, including various metal oxides and carbon-based catalysts. Ultimately, for MFC cathodes to enhance power output, they must efficiently reduce overpotential and accelerate ORR kinetics.

#### b. Reaction selectivity

At the cathode, molecular oxygen undergoes electrochemical reduction via either a two-electron ( $2e^-$ ) pathway producing hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), or a four-electron ( $4e^-$ ) pathway yielding water ( $\text{H}_2\text{O}$ ) in aqueous systems (N. Wang et al. 2021). These pathways typically compete, though the  $4e^-$  route is generally preferred in oxygen reduction reactions (ORR), as  $\text{H}_2\text{O}_2$  generated via the  $2e^-$  route may alter electrolyte

properties and suppress microbial activity on biocathodes. Therefore, reaction selectivity becomes a critical parameter in designing BES cathode materials, as it enhances catalytic efficiency by promoting the desired pathway (Exner. 2023). Another challenge arises with air-exposed cathodes, particularly regarding the stability and permeability of the materials. Metal catalysts like Cu and Ni are prone to leaching into the anolyte (Qiu et al. 2021), posing toxicity risks to biofilms and leading to catalyst layer degradation. This not only inhibits ORR kinetics but also reduces the overall energy output. Consequently, cathode materials must exhibit high catalytic performance while retaining structural stability to ensure long-term BES functionality.

This preference for the four-electron ORR pathway is closely linked to the reaction mechanism occurring at the cathode surface. The oxygen reduction reaction proceeds through a series of surface-bound intermediates, including superoxide ( $O_2^-$ ), hydroperoxyl ( $OOH^*$ ), atomic oxygen ( $O^*$ ), and hydroxyl species ( $OH^*$ ), whose adsorption and transformation are governed by the surface chemistry and electronic structure of the cathode material (Zhang et al. 2017; Zhi et al. 2021). In BES sensors, materials that enable effective O–O bond cleavage and maintain balanced adsorption of oxygenated intermediates favor complete oxygen reduction to water, thereby supporting stable cathodic potentials and consistent signal output (Rong et al. 2025). In contrast, cathode materials with unfavorable surface energetics tend to stabilize peroxide-related intermediates, shifting the reaction toward the two-electron pathway and promoting hydrogen peroxide formation. Such peroxide accumulation is undesirable in BES sensors as it can diffuse into the electrolyte and progressively suppress electroactive microbial activity (Merino-Jimenez et al. 2021), ultimately compromising sensor reliability and long-term performance. Lastly, cathode material selection must account not only for ORR activity but also for intrinsic reaction selectivity to ensure biologically compatible and durable BES operation.

## 2.4 INTERNET OF THINGS (IoT) IN SENSOR TECHNOLOGY

The integration of the Internet of Things (IoT) in sensor technology has significantly enhanced data collection, analysis, and real-time decision-making across various industries (Krishnamurthi et al. 2020; Qinxia et al. 2021). IoT-integrated systems utilize

interconnected networks, such as cloud services, to provide continuous monitoring, automation, and allow users access the data at any time from different locations (Garrido-Momparler et al. 2022). This progress is especially notable in areas like environmental monitoring (Garrido-Momparler et al. 2022), industrial automation (Haghnegahdar et al. 2022; Priyanka et al. 2021), and smart infrastructure (Eifert et al. 2020), where sensors gather vital data and send it to cloud platforms for analysis. Two significant uses of IoT in sensor technology are cloud-based data streaming and real-time alert systems, both of which are essential for saving cost with unmanned supervision, boosting operational safety and efficiency, and improving the decision-making speed.

#### **2.4.1 Cloud data streaming for real-time monitoring**

Cloud data streaming enables real-time data acquisition by transmitting sensor data to cloud-based platforms, where the data can be accessed and analyzed remotely by all users (Gulati et al. 2022). In IoT-based sensor networks, data is continuously collected from the sensors installed in various environments, such as water quality monitoring systems (AlMetwally et al. 2020), air pollution detectors (Kaivonen et al. 2020), industrial process controls (McCann et al. 2021), and smart agricultural applications (Khanal et al. 2020). Instead of manual data collection by operators, cloud-based streaming allows data to be transmitted in real time to centralized platforms, where it is analyzed using advanced algorithms, artificial intelligence, or machine learning (Andronie et al. 2021; Priyadarshi. 2024).

One of the main advantages of cloud data streaming is its ability to handle large volumes of data generated by IoT sensors without significant delays. In water quality monitoring applications, operators can deploy multiple sensing probes connected with cloud service to constantly measure the physicochemical parameters such as temperature, pH, turbidity, conductivity, dissolved oxygen (DO), total dissolved solids (TDS), and total suspended solids (TSS) (Butt et al. 2022; Sagan et al. 2020). These sensors continuously send real-time data to cloud servers, allowing operators to make informed decisions and take preventive measures before costly breakdowns occur. However, parameters like chemical oxygen demand (COD) and biochemical oxygen

demand (BOD) still require conventional lab-based chemical analysis, which is performed offsite, costly, time consuming and poses a major challenge in real-time online sensing. To address this issue, BES-based sensors with rapid feedback through microbial metabolism and are connected to a cloud service can serve as real-time COD and BOD sensors (Lu et al. 2022; Sonawane et al. 2020).

Cloud-based platforms also facilitate remote access and data sharing among multiple users. By integrating cloud services with mobile applications or web-based dashboards, real-time sensor data can be visualized in graphical form and viewed by users freely at any time. This function is particularly valuable in critical applications such as water quality monitoring, where operators can remotely monitor the incoming influent without travelling all the way to the treatment plant. Furthermore, cloud-based data storage allows for historical data analysis, trend prediction, and improved decision-making, which can be upgraded by including a notification system (Wu et al. 2020).

#### **2.4.2 Real-time notification systems for instant alerts**

In addition to real-time data streaming using cloud services, IoT-based sensor can be incorporated with notification systems to alert users when a preset threshold is exceeded or anomalies are detected (Poornima et al. 2020). This is particularly important in applications where immediate action is required to prevent equipment damage, safety hazards, or failures. IoT-enabled alarms and alerts can be configured to notify users via multiple channels, including email, mobile app notifications, or even local on-site ringing device.

According to Sean et al. (2020), a supervisory control and data acquisition (SCADA) system coupled with water quality sensors was installed in a wastewater treatment plant to remotely monitor energy consumption and water quality. The authors reported that the data collected by the system were used for simulation and prediction, which helped to conserved energy during wastewater treatment as well as preventing damage to the facility (Sean et al. 2020). This indicates that the rapid response from the sensors coupled with a notification system enhances safety by ensuring immediate awareness and action, especially in wastewater treatment plants (Karn et al. 2023). In

other industrial settings, IoT sensors deployed in factories and power plants continuously monitor critical parameters such as temperature, pressure, gas leakage, and vibration levels (Jo et al. 2020; Kralovec et al. 2020). When an abnormal reading is detected, the system triggers an alert and automatically notifies relevant personnel via cloud-based notification services (Esposito et al. 2022).

## **2.5 MATHEMATICAL MODELS IN SENSOR TECHNOLOGY**

Mathematical models play a critical role in converting the raw electrical signals from microbial electrochemical sensors into useful information for water quality monitoring. Rather than focusing on complex electrochemical or biological kinetics, this study employs a signal processing approach based on anomaly detection and change point detection to analyze the sensor's real-time output. These models are essential for identifying deviations from normal operating conditions and pinpointing the exact moment a pollution event or system failure occurs.

### **2.5.1 Anomaly detection: Identifying outliers**

Anomaly detection is a model used to identify data points that do not conform to an expected pattern or behavior within a dataset (Erhan et al. 2021; Martí et al. 2015). For MESe and MFCSe systems, this involves establishing a baseline during normal condition and triggering an alarm when any signal falls outside of this range. A common and straightforward application of this model is through a threshold-based method, where upper and lower limits are set for the sensor's voltage or current output (Yu et al. 2020).

A practical application is in the detection of toxic shock loads in industrial wastewater (Oliveira et al. 2023). Recent studies have highlighted the growing importance of real-time anomaly detection using threshold alarms and dynamic baselining in wastewater quality monitoring. Ghinea et al. (2023) demonstrated the effectiveness of integrating smart sensors with threshold-based models, where anomalies were first flagged using predefined limits for parameters like pH, conductivity, and dissolved oxygen. These rapid detections were then validated by

machine learning algorithms such as Random Forest, which achieved up to 98.53% accuracy, offering both speed and reliability in pollution alert systems. However, fixed thresholds can sometimes fail to account for natural fluctuations in wastewater composition (Ghinea et al. 2023). To address this, Navato and Mueller (2021) introduced a data-driven approach using K-means clustering to establish behavioral baselines over 17 months of spectral data. This method allowed more flexible alarm triggers by grouping similar patterns of influent composition, thereby reducing false positives and improving responsiveness to sudden changes, such as those observed during the COVID-19 pandemic. By combining static thresholds with adaptive clustering, these studies support a hybrid model for real-time monitoring that balances simplicity and computational efficiency with improved detection accuracy (Navato et al. 2021). In short, such approaches are highly valuable for decentralized or IoT-enabled systems, where energy-efficient, low-latency alarms are critical for timely intervention and operational reliability in wastewater treatment plants.

### **2.5.2 Change point detection: Identifying onset of change**

While anomaly detection is effective for flagging a signal outside a threshold, change point detection is a more sophisticated model that is used to identify the exact time at which the statistical properties of a signal change. This model focuses on the rate of change of the sensor's output, rather than just its absolute value. This is particularly useful for detecting more subtle or gradual changes in water quality that may not immediately trigger a threshold-based anomaly, specifically useful for signal spikes. The model analyzes the rate of change of the sensor's signal real-time.

Changepoint detection has become increasingly crucial for environmental monitoring, particularly in scenarios involving real-time data monitoring. Militino et al. (2020) proposed a robust statistical framework to detect multiple changepoints in environmental time series, such as rainfall and temperature using Bayesian models with penalized cost functions. This approach enables accurate localization of abrupt changes while handling seasonal patterns and noise, which was highly relevant for changepoint detection in dynamic systems (Militino et al. 2020). This work emphasized the need for continuous, data driven detection strategies, which are statistical modeling versus

remote sensing-based observation. A significant research gap remains in translating such methodologies into real-time and field-deployable platforms like bioelectrochemical sensors. There is no study directly addresses real-time changepoint detection in biosensing applications or the use of rate-of-change (RoC) metrics for triggering alarms. Future work should aim to bridge these domains by integrating statistical changepoint detection with real-time biosensor signal processing. Additionally, deploying these systems via IoT and cloud frameworks would enhance their responsiveness and scalability for smart wastewater infrastructure and real-time pollution alerts.

## 2.6 SUMMARY OF RESEARCH GAP

Despite extensive advancements in microbial electrochemical technologies for environmental monitoring, several gaps can be determined that limit their widespread practical application. Firstly, while many studies have demonstrated the feasibility of MESe in laboratory settings, limited research has addressed the optimization of surface-modified electrodes for enhancing signal sensitivity and microbial adhesion in real-time wastewater conditions. Secondly, although MFCSe offer energy-efficient, self-sustaining operation, most configurations still rely on conventional submerged cathodes, which are prone to fouling and require external power input. The development and validation of tubular-type air-cathode MFCSe with membrane-electrode assembly design remains underexplored, especially under fluctuating wastewater conditions.

Moreover, the integration of IoT frameworks into bioelectrochemical systems is emerging but is still lacking in terms of applicability. The incorporation of mathematical models such as rate-of-change (RoC) and threshold detection algorithms for triggering automated notifications is still scarce, especially for real-time monitoring applications. The lack of standardized protocols for signal baselining and real-time anomaly detection continues to limit their utility in industrial monitoring applications. Lastly, although short-term laboratory validations are common, long-term performance evaluations of MFCSe systems in real-world municipal wastewater treatment plants are scarce. This research addresses these gaps by combining electrode engineering, sensor architecture,

algorithmic integration, and field deployment to create a comprehensive biosensing platform for intelligent wastewater monitoring.



## **CHAPTER III**

### **METHODOLOGY**

#### **3.1 INTRODUCTION**

This chapter systematically outlines the methodology used in this work. In general, the main purpose of this work is to provide an alternative solution for rapidly analyzing water quality through the detection of pollutant, which can be further serve as a cost-effective water quality monitoring tool. To address the objectives mentioned in Chapter 1, this study is divided into three phases, namely, (i) the design and development of bioelectrochemical sensors, (ii) validation of the sensors through pollutants testing, and (iii) the integration of alarm notification. As mentioned in Chapter 1, two types of bioelectrochemical sensors.

Figure 3.1 outlines the workflow of this study, directly aligning with the research objectives. The process begins with the design and construction of the microbial electrochemical sensor (MESe) and microbial fuel cell sensor (MFCSe), which involves the fabrication and characterization of surface-modified electrodes. Following sensor assembly, pollutant testing is conducted to evaluate their sensitivity, stability, and robustness under various simulated wastewater conditions. In the subsequent phase, an Internet of Things (IoT)-enabled system is integrated with the sensors, incorporating mathematical models to facilitate real-time data processing and automated alarm notifications. The final stage involves the long-term field deployment of the system at a municipal wastewater treatment plant to assess its signal stability, performance, and operational reliability under real-world conditions.

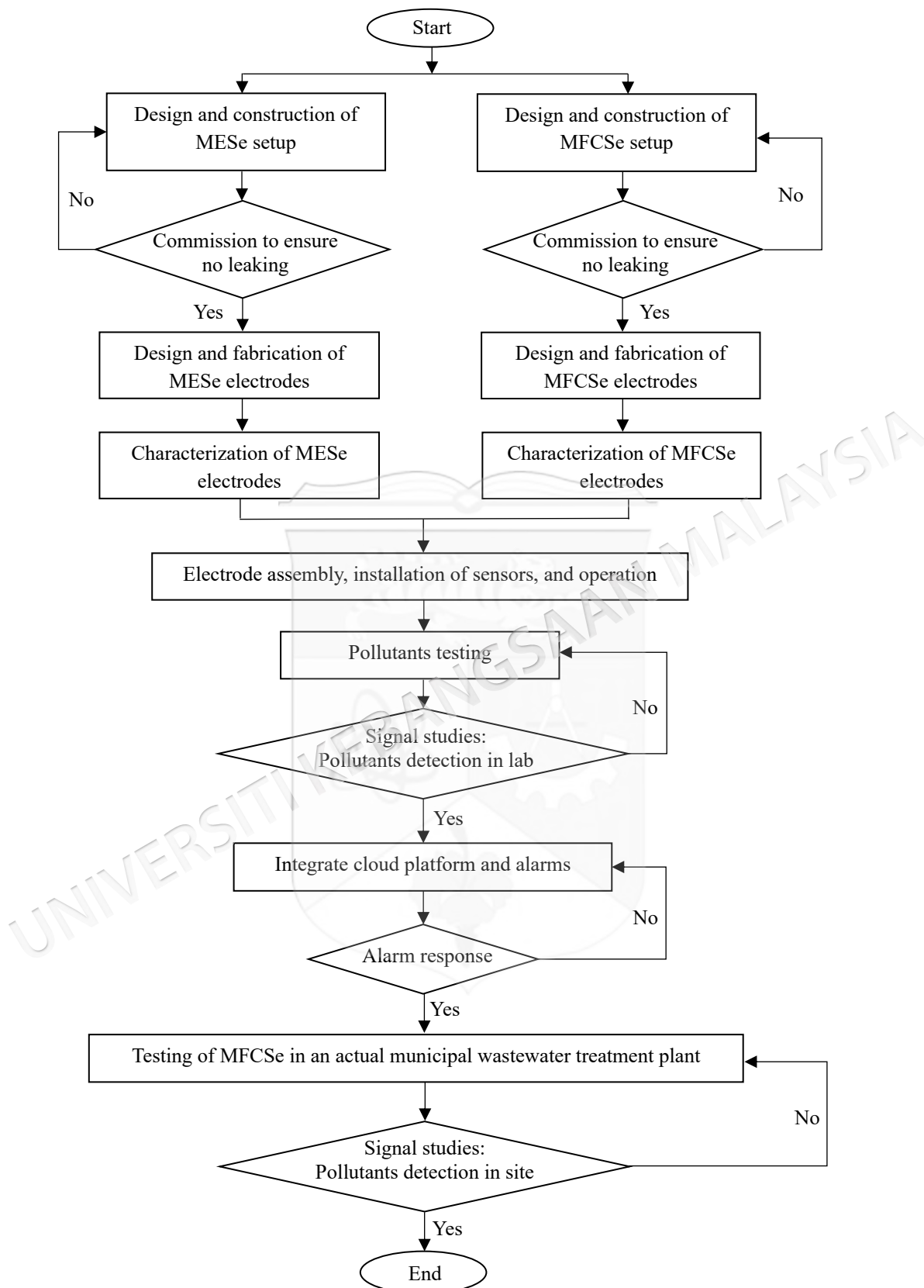


Figure 3.1 Research flowchart of this study.

### 3.2 LIST OF CHEMICALS AND MATERIALS

The chemicals and materials used in this study are listed in Table 3.1.

Table 3.1 List of materials and chemicals used in this study.

| Chemical                               | Specification                | Supplier                     |
|----------------------------------------|------------------------------|------------------------------|
| 4-nitrophenol, $C_6H_5NO_3$            | AR grade                     | Fisher Scientific            |
| Ammonium chloride, $NH_4Cl$            | AR grade, 99.8%              | System                       |
| Ammonium persulfate, $(NH_4)_2S_2O_8$  | ACS grade, 98%               | Sigma-Aldrich                |
| Aniline, $C_6H_5NH_2$                  | USP grade, 99.9%             | Sigma-Aldrich                |
| Carbon black, C                        | Vulcan XC 72R                | Fuel Cell Store              |
| Ethyl alcohol, $C_2H_5OH$              | ACS grade, 99.5%             | Sigma-Aldrich                |
| Hydrochloric acid, HCl                 | AR grade, 37%                | Fisher Scientific            |
| Isopropyl alcohol, $C_3H_7OH$          | ACS grade, 99.5%             | Sigma-Aldrich                |
| Malathion 57                           | AR grade                     | Merck                        |
| Methyl orange, $C_{14}H_{14}N_3NaO_3S$ | ACS grade, 85%               | Sigma-Aldrich                |
| Nafion 117 solution                    | 5 wt. %                      | Sigma-Aldrich                |
| Nitric acid, $HNO_3$                   | ACS grade, 70%               | Sigma-Aldrich                |
| Platinum on carbon, PtC                | Vulcan XC-72, 20%            | Fuel Cell Store              |
| Polytetrafluoroethylene, PTFE          | 60 wt. % dispersion in water | Sigma-Aldrich                |
| Polyvinyl alcohol, PVA                 | 99% hydrolyzed               | Sigma-Aldrich                |
| Potassium hydroxide, KOH               | AR grade, 90%                | Sigma-Aldrich                |
| Sodium acetate, $CH_3COONa$            | ACS grade, 99.9%             | Merck                        |
| Sodium hydroxide, NaOH                 | ACS grade, 98%               | Sigma-Aldrich                |
| Thinner                                | DL 1511                      | Socomore                     |
| Yeast extract powder                   | For microbiology use         | Merck                        |
| Ag/AgCl reference electrodes           | 3M KCl                       | Sigma-Aldrich                |
| Carbon cloth                           | AvCarb 1071 HCB              | Fuel Cell Store              |
| Cation exchange membrane               | CMI-7000                     | Membranes International Inc. |
| Stainless steel                        | SS304L grade                 | Kok Hoo Steel                |

### 3.3 INSTRUMENTATIONS

The instruments used in this study are elaborated in Table 3.2.

Table 3.2 List of instruments used in this study.

| Instrument                                                                                        | Model                              | Function                                                                                   |
|---------------------------------------------------------------------------------------------------|------------------------------------|--------------------------------------------------------------------------------------------|
| 4-channel Potentiostat                                                                            | Quadpot, Whistonbrook Technologies | To perform electrochemical studies on the sensors                                          |
| Potentiostat (AUTOLAB)                                                                            | PGSTAT128N, Metrohm                | To perform electrochemical studies on the electrodes                                       |
| Field emission scanning electron microscope with energy dispersive X-ray spectroscopy (FESEM-EDX) | Supra 55VP, ZEISS                  | To inspect the surface morphology and identify the elemental information of the electrodes |
| Raman spectrometer (RAMAN)                                                                        | DXR2xi, Thermo Scientific          | To determine the molecular functional groups on the electrodes                             |
| Multiparameter pH meter                                                                           | HI2020-02 edge, Hanna              | To measure pH, conductivity, and temperature of samples                                    |
| Laser cutting machine                                                                             | ST-FC3015FM, StyleCNC              | To laser cut metal plates                                                                  |
| Oven                                                                                              | UF110, Memmert                     | To dry materials                                                                           |
| Muffle furnace                                                                                    | FB1410M, Fisher Scientific         | To sinter materials                                                                        |
| Ultrasonic bath                                                                                   | Elmasonic P, Elma                  | To homogenize a mixture of solutions                                                       |
| Vacuum oven                                                                                       | DZF-6020                           | To dry materials                                                                           |
| DC power supply                                                                                   | E3631A, Keysight Technologies      | To provide a stable voltage                                                                |
| Precision data logger                                                                             | ADC-24, Pico Technology            | To measure and record data over time                                                       |

### 3.4 DESIGN AND DEVELOPMENT OF MICROBIAL ELECTROCHEMICAL SENSOR (MESe)

#### 3.4.1 Design and construction of MESe setup

The design of the MESe water circulation setup (Figure 3.2) was drawn using computer aided design (CAD) software. Four two-electrode MESeSes were included in this design to compare the performances of different surface modified MESe anodes. Tee-fittings were used to hold and fix the MESeSes in place. A flowmeter was placed at the inlet to constantly display the flowrate of the incoming water. A static mixer was placed within the pipelines to induce turbulence for continuous mixing, which prevented sludge agglomeration and blockages. A sampling port was installed after the MESeSes to collect sample for analysis.

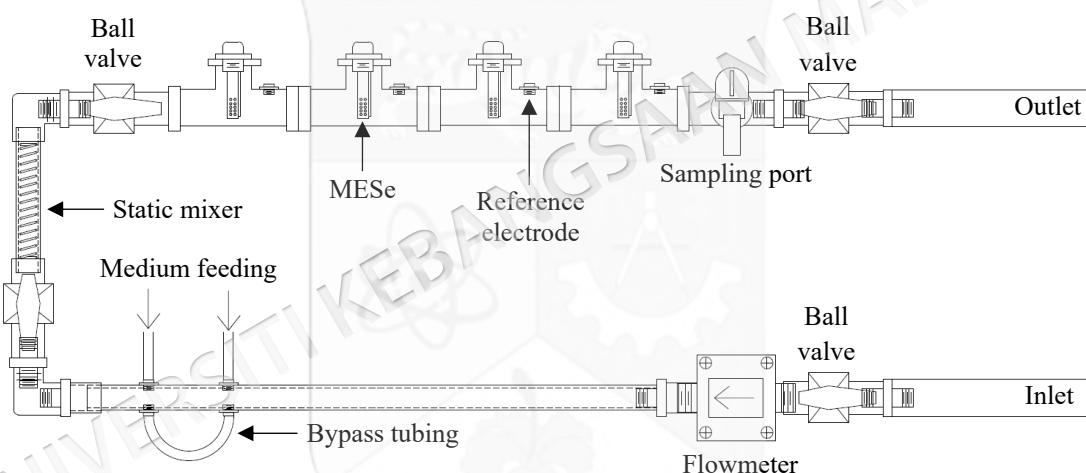


Figure 3.2 Schematic illustration of the MESe setup.

The materials needed to construct this setup is listed in Table 3.3.

Table 3.3 Materials for water circulation setup construction.

| Materials            | Function                                        |
|----------------------|-------------------------------------------------|
| Ball valve           | To control the flow of water                    |
| Coupling and fitting | To connect the PVC pipes with other components  |
| Peristaltic pump     | To provide feeding of mediums                   |
| Push-in fitting 5 mm | To insert reference electrode                   |
| PVC pipe 25 mm       | Setup main body frame                           |
| PVC solvent cement   | To bond PVC pipes with other pipes and fittings |

... to be continued

... continuation

|                                 |                                          |
|---------------------------------|------------------------------------------|
| Silicone sealant                | To seal any openings to prevent leakage  |
| Transparent plastic tubing 3 mm | To inject medium and serve as the bypass |
| Water pump                      | To provide circulation for the setup     |
| Wi-Fi socket timer              | To control the pumps remotely            |

### 3.4.2 Design and fabrication of MESe electrodes

Stainless steel 304L (SS304L) was selected as the material for both electrodes and their design is illustrated in Figure 3.3. All SS304L electrode surfaces were polished with commercially available 600 grit sandpaper to remove any impurities. The polished electrodes were used as cathodes as well as anode for the control sensor. Apart from the control anode, three other anodes were prepared with different surface modifications, namely, (i) carbon black (CB)-coated, (ii) flame oxidised (FO), and (ii) acid bleached (AB). Carbon ink was prepared by sonicating a mixture 100 mg of CB powder, 500 mg of Nafion solution, and 500 mg of isopropanol for 20 minutes. The as-prepared carbon ink was then spray coated onto the polished SS304L electrode to yield CB-anode. FO-anode was prepared by heating the polished SS304L electrode directly over a Bunsen burner for 2 minutes until a red-hot condition was observed, then it was allowed to cool under room temperature conditions. To fabricate the AB-anode, a polished SS304L electrode was immersed in aqua regia (prepared by mixing  $\text{HNO}_3$  and  $\text{HCl}$  with a ratio of 1:3) for 12 hours to remove any metal residues from the electrode surface. Then, the electrodes were rinsed with deionized water and dried in an oven.

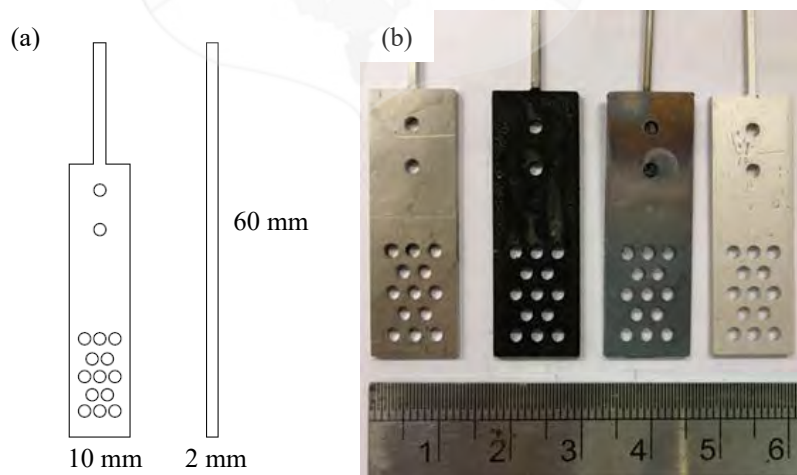


Figure 3.3 Electrode design of MESe (a) schematic drawing and (b) actual electrode coated with catalysts (from left to right: control, carbon-coated, flame oxidised, and acid bleached).

### 3.4.3 Assembly of electrodes and installation of MESe

The MESe anode and cathode were arranged side by side and fitted into a PVC plug end cap. The cap was pre-drilled with two holes to allow the electrodes to be connected to electrical wires. Then, the electrodes were fixed in place by pouring mixed epoxy formula (3 parts epoxy resins and 1 part epoxy harder) into the cap opening and allowed to cure for 24 hours. Finally, the assembled MESe can be conveniently installed into the tee-fitting as shown in Figure 3.4.

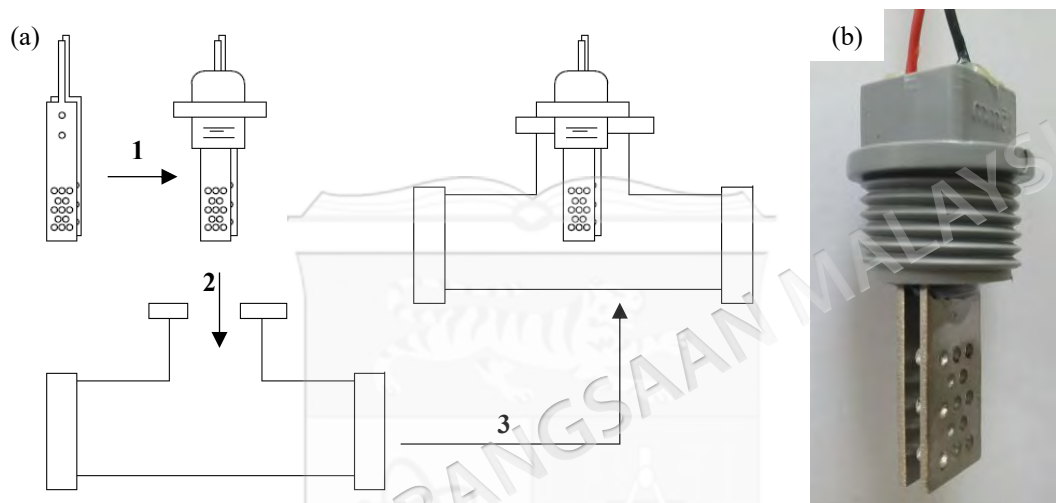


Figure 3.4 MESe electrodes assembly (a) schematic drawing and (b) actual assembled MESe.

### 3.4.4 Physicochemical characterization of electrodes

The surface morphology of the modified electrodes was characterized using field emission scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (FESEM–EDX; Supra 55VP, ZEISS, Germany). Elemental mapping was performed to detect the presence of Fe, C, O, Cr, Ni, Mn, Si, F, and S. All samples were examined at a magnification of 300 $\times$  to observe microstructural features. Surface morphology and roughness were analysed using atomic force microscopy (AFM NTEGRA Prima, NT-NDT, Moscow, Russia). Topographical scans were collected over a selected 10  $\times$  10 nm region of the sample surface to characterise nanoscale features. The acquired height images were further processed through line profile analysis to extract quantitative roughness parameters, including minimum and maximum heights, mean surface level, peak-to-valley distance, and average roughness. Additionally, Raman spectroscopy (RDXR2xi, Thermo Scientific, USA) was employed to analyze

the surface chemistry in real time. Samples were placed on the imaging stage, and regions of interest were selected for spectral acquisition. Raman spectra were recorded in the range of 3500 to 0  $\text{cm}^{-1}$ , with intensity plotted as a function of Raman shift.

### 3.4.5 Electrochemical characterization of electrodes

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) analyses were performed after the BES biosensors were enriched and had achieved signal stability. EIS was carried out using a potentiostat (PGSTAT128N, Metrohm, Switzerland) with a frequency response analyzer (FRA) module to obtain the internal polarization ( $R_p$ ) and solution ( $R_s$ ) resistances. The results generated from the EIS were analyzed with NOVA control software (Metrohm, Switzerland). Three spectrograms were recorded from each biosensor, which were between: (1) anode and cathode, (2) anode and reference electrode, and (3) cathode and reference electrode. Ag/AgCl was used as the reference electrode for all biosensors in this study. All potentials applied or recorded by the digital multimeter were referenced to the standard hydrogen electrode (SHE) by adding the constant RE potential of +0.2 V vs. SHE. CV was performed using a 4-channel potentiostat (QuadPot, Whistonbrook Technologies, UK) to examine the bio-electrocatalytic activity of the MFC biosensors. CVs were carried out from -0.8 V to +0.4 V at a scan rate of 5  $\text{mV s}^{-1}$  for 3 cycles. EIS and CV were performed without connecting the biosensors' external resistors.

### 3.4.6 Startup, operation, and enrichment

MESe and MFCSe share similar startup procedures and operational methods. Prior to sensor installation, the setup was commissioned for a week with tap water to identify any water leakage or improper fitting connections. The flowrate used for all setups were fixed at 15  $\text{L min}^{-1}$  using the same brand of water pump to circulate water from a 25 L reservoir tank. The initial synthetic wastewater was prepared by adding 0.25  $\text{g L}^{-1}$  of yeast extract. A peristaltic pump controlled by a Wi-Fi socket timer was installed to inject medium into the system during nutrient feeding and pollutant testing. The feeding medium consisted of 0.25 M sodium acetate,  $\text{CH}_3\text{COONa}$  and 0.125 M ammonium chloride,  $\text{NH}_4\text{Cl}$  and was injected to the setup every 12 hours for microbial consumption.

and biofilm development on the electrode. The inoculum used in the system was cultured from a water sample collected at the Universiti Kebangsaan Malaysia (UKM) engineering faculty's lake and enriched with an iron-rich nutrient medium. Ag/AgCl (3.0 M KCl) were used as reference electrode and placed near to each sensor. A multichannel precision data logger (ADC-24, Pico Technology) was connected to each sensor and its reference electrode to measure the voltage, half-cell potential, and current at 1 minute intervals. A direct-current (DC) power supply (E3631A, Keysight Technologies) was used to provide a fixed voltage to the MESes, while MFCSe were not connected to any external power supply. Lastly, duplicates of MESe and MFCSe setups were constructed.

### 3.4.7 Pollutants testing

Pollutants testing (Table 3.4) was carried out after the sensors were enriched to identify the sensing performance of MESes and MFCSe. A series of model pollutants, namely, sodium acetate ( $\text{CH}_3\text{COONa}$ ), ethanol, thinner, palm oil mill effluent (POME), 4-nitrophenol, malathion, and methyl orange were tested using a novel test sequence designed for this research. Firstly, the incremental test was performed by injecting 100 mL of pollutants at concentration of  $100 \text{ mg L}^{-1}$  every hour for up to 8 hours. This was followed by the dumping test, where 1 L of pollutants was fed to the system all at once. Lastly, water dilution test was performed by draining the reservoir to half its volume and filling it with 10 L of fresh tap water. The signal responses were monitored and recorded during the incremental, dumping, and water dilution tests.

Table 3.4 Pollutant testing sequences and their purpose.

| Test                       | Purpose                                                                                               |
|----------------------------|-------------------------------------------------------------------------------------------------------|
| Pollutant incremental test | To analyze the sensor's response and sensitivity to a small volume of pollutant at different periods. |
| Pollutant dumping test     | To determine the sensor's robustness by introducing a high volume of pollutant.                       |
| Water dilution test        | To simulate a rainy condition similar to Malaysia's weather.                                          |

### 3.4.8 Liquid sample analysis

Liquid samples were collected 10 minutes after every pollutant injection during the incremental and dumping tests, as well as after fresh tap water was added during the water dilution test. Approximately 12 mL of samples were collected from the setup's sampling port and filtered with 0.45  $\mu\text{m}$  syringe filters before analysis. The temperature, conductivity, and pH values of the samples were measured and recorded using a benchtop multiparameter pH meter. Chemical oxygen demand (COD) values were determined using COD analysis kits (HACH, USA). Total nitrogen-ammonium ( $\text{N-NH}_4^+$ ) analysis was only performed for 4-nitrophenol. A UV-VIS spectrophotometer (UV-1900i Plus, Shimadzu, Japan) was used for measuring the concentration of methyl orange dye *via* absorbance. Total phosphate test (HACH, USA) was carried out for liquid samples tested against malathion.

## 3.5 DESIGN AND DEVELOPMENT OF MICROBIAL FUEL CELL SENSOR (MFCSe)

### 3.5.1 Design and construction of MFCSe setup

Figure 3.5 (a) shows the design of the MFCSe setup using the same CAD software. Similar concepts were applied in this design, including the incorporation of flowmeter, ball valves, sampling port, bypass tubing, and static mixer. The main difference between the MFCSe and MESe setups lies in the configuration of the sensors. Instead of using a tee-fitting, eight pipe openings were drilled to allow direct contact between the sensor anode and the flowing water, as shown in Figure 3.5 (b-c). Similar plumbing materials were used as in Table 3.3.

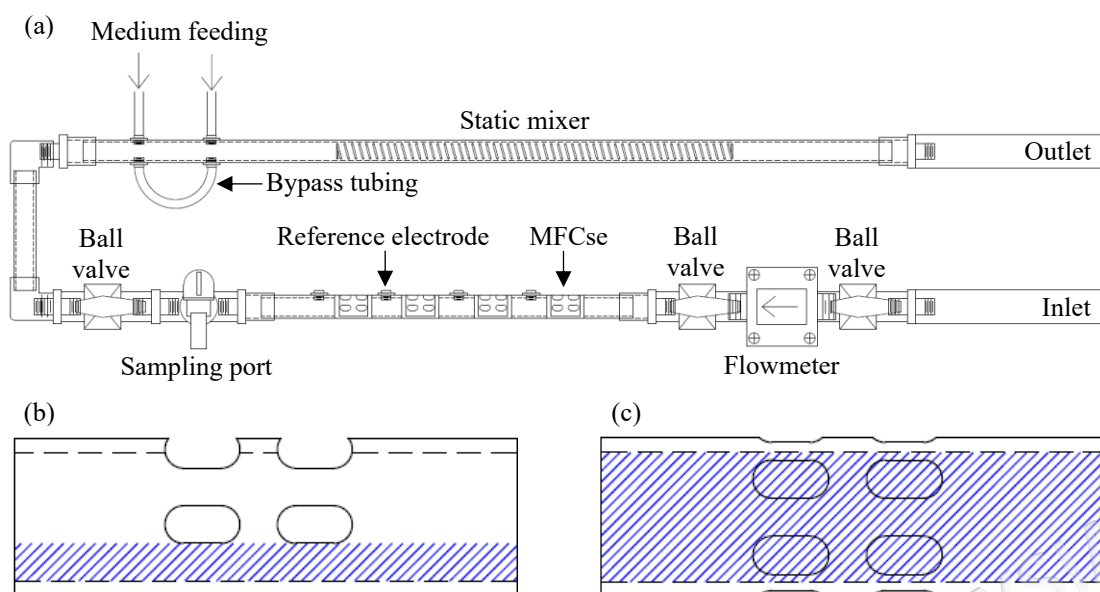


Figure 3.5 Schematic illustration of (a) MFCSe setup, (b) side view and (c) top view of the pipe openings for the sensor.

### 3.5.2 Design and fabrication of MFCSe electrodes

Carbon cloth (CC) with a size of  $12 \text{ cm}^2$  was used as the material for both electrodes and separated by a cation exchange membrane (CEM). Plain CCs were pretreated with 99.5% ethanol solution for 30 minutes, followed by 1.0 M HCl for 1 hour, 1.0 M NaOH solution for 1 hour, and finally rinsed with deionized water. The pretreated CCs were used as anodes. Four types of MFCSe air-cathodes were fabricated in this work with different modifications, namely, (i) polytetrafluoroethylene (PTFE)-coated, (ii) PTFE/carbon black (PTFE/CB)-coated, (iii) PTFE/CB/20% Platinum on carbon (PTFE/CB/PtC)-coated, and (iv) PTFE/CB/polyaniline (PTFE/CB/PANI)-coated. PTFE air-cathode served as the control in this work and it was prepared by coating the pretreated CC with 30 wt.% PTFE solution and sintered in a furnace at  $350^\circ\text{C}$  for 30 minutes. PTFE/CB was prepared by adding  $2.5 \text{ mg cm}^{-2}$  CB powder on one side of the PTFE-coated CC and sintered in a furnace at  $350^\circ\text{C}$  for 30 minutes. PtC ink was prepared by suspending the PtC powders in a mixture solution with a ratio of 1 (20% PtC in mg): 0.8 (deionized water): 6.5 (Nafion): 3.5 (isopropanol) and ultrasonicated for 30 minutes. Then, the PtC ink was evenly applied on the CB-coated surface of PTFE/CB and dried in an oven at  $70^\circ\text{C}$  for 12 hours to yield PTFE/CB/PtC. PANI ink was prepared by mixing 10 ml of aniline with 350 ml of 2.0 M HCl and stirred for 12 hours, followed by adding ammonium persulfate (APS) dropwise until the solution

changes color from colorless to dark green was observed indicating the polymerization process has completed. The resultant polymer was vacuum filtered and rinsed with 2.0 M HCl and deionized water before drying in a vacuum oven at 70 °C for 24 hours. PTFE/CB/PANI air-cathode was prepared by coating a mixture solution of PANI powders ( $0.5 \text{ mg cm}^{-2}$ ) and 100  $\mu\text{l}$  polyvinyl alcohol (PVA) on the CB-surface of PTFE/CB and dried in an oven at 70 °C for 12 hours.

### 3.5.3 Assembly of electrodes and installation of MFCSe

The MFCSe was assembled using a membrane-electrode assembly (MEA) configuration (Figure 3.6). The air-cathode was exposed to atmospheric environment while the anode remained in contact with the water. This was achieved by wrapping the pre-treated plain CC anode around the pipe openings, followed by the CEM as the separator, and finally the modified air-cathode with the catalyst side facing the CEM to improve ionic mass transfer. Then, the MFCSe was secured with cable ties and sealed with a silicone sealant to prevent any leakage.

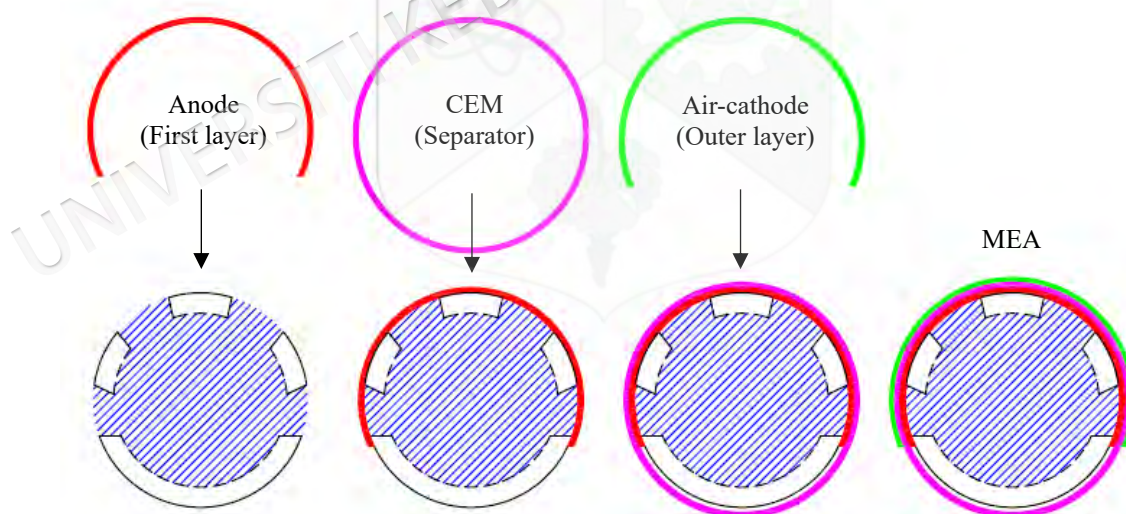


Figure 3.6 MFCSe electrodes assembly using a MEA configuration.

The upcoming methodologies for MFCSe are similar to those of MESe from subsections 3.4.4 to 3.4.8.

### 3.6 INTEGRATION OF ALARM NOTIFICATION

Two mathematical models were proposed as the detection method: change point detection and anomaly detection methods. Change point detection methods play a crucial role in identifying anomalies in data patterns, which allows the detection of small or significant changes in a time series. One approach to change point detection involves analyzing the rate of change, represented by the derivative  $dy/dx$ , to pinpoint abrupt transitions from one point data to the next in a time series. By examining the rate of change at different points in the dataset, anomalies or structural shifts can be identified, indicating potential change points such as spikes. This method is particularly useful in scenarios where sudden variations or disruptions occur in the data, allowing for the timely detection of critical events or deviations from the expected pattern. Analyzing the rate of change provides valuable insights into the dynamics of the data, facilitating the identification of change points and enhancing the understanding of underlying trends and patterns within the dataset. The equation (Equation 3.1 and Equation 3.2) below shows the mathematical equations used for the change point detection:

$$\text{Equation of a line : } y = mx + C, \text{ where } y = A \text{ m}^{-2} \text{ and } x = \text{min} \quad (3.1)$$

$$\text{Derivative : } \frac{dy}{dx} = m, \text{ where } \frac{dy}{dx} = (A \text{ m}^{-2})/\text{min} \quad (3.2)$$

Anomaly detection based on maximum and minimum thresholds is usually applied in monitoring systems to identify deviations from a certain range. By setting thresholds for acceptable ranges of data, anomalies can be detected when data points fall outside these predefined limits. This method involves establishing upper and lower boundaries that define normal operating conditions, allowing for the timely identification of unusual patterns or outliers in the data. Anomaly detection based on maximum and minimum thresholds provides a straightforward and intuitive way to flag anomalies, making it easier to interpret and respond to unexpected changes in the system.

### 3.7 MFCSE FOR ACTUAL MUNICIPAL WASTEWATER TREATMENT CONDITIONS

#### 3.7.1 Fabrication of pilot scale MFCSe for site

Figure 3.7 illustrates the design and assembly of the biosensor system. To enable electrode interaction with the water inside the pipeline, two holes were drilled into the pipe body. A transparent double-sided tape was then affixed to the surface of the pipe, with openings cut to match the drilled holes, ensuring proper alignment and sealing (Figure 3.7 (b)(i)). A plain carbon cloth was selected as the anode material, positioned as the first layer in direct contact with the wastewater (Figure 3.7 (b)(ii)). This layer plays a crucial role in facilitating microbial colonization and initial electron transfer. Next, a cation exchange membrane (CEM), sized to match the dimensions of the double-sided tape, was layered over the anode. The CEM acts as a separator between the anode and cathode, allowing selective ion transfer while preventing oxygen diffusion to the anode side (Figure 3.7 (b)(iii)). To ensure independent operation and avoid electrical interference, a minimum spacing of 5 mm was maintained between each sensor unit. Four individual sensors were assembled and housed within a 2-inch diameter pipe (Figure 3.7 (b)(iv)). Following the CEM installation, a modified air-cathode was added as the third layer. The catalyst-coated side of the cathode was oriented to face the CEM to optimize redox reactions and ion transport across the membrane. Two titanium plates were embedded as current collectors to enhance electron conduction (Figure 3.7 (b)(v)). Finally, the fully assembled sensor units were secured tightly using cable ties to prevent any potential leakage or misalignment during operation (Figure 3.7 (b)(vi)).

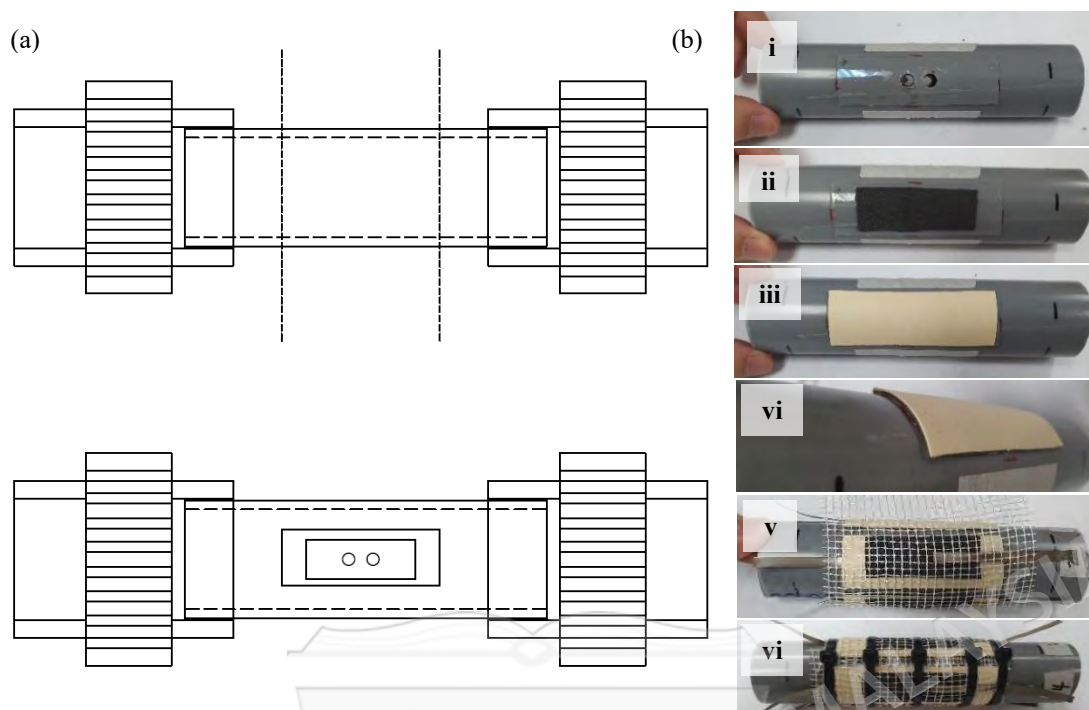


Figure 3.7 (a) Design and (b) Construction of MECSe biosensor for actual municipal wastewater quality detection.

### 3.7.2 Installation and application

The tubular-type multiarray biosensor was installed at a test site located adjacent to a raw sewage tank at Indah Water Research Centre (IWRC) located in Titiwangsa, Kuala Lumpur, Malaysia. The primary objective of this installation was to monitor the characteristics of raw sewage directly supplied from a local sewage treatment plant (STP). Figure 3.8 shows the biosensor setup and site installation, illustrating the sensor placement, sewage circulation system, and data acquisition components. To facilitate continuous flow through the biosensor, an aquarium pump (BO-14000A, SOBO, China) was installed, enabling the circulation of sewage through the sensor chamber. Electrical cables were connected from the biosensor's anode and cathode electrodes to a data logger (ADC-24, Pico Technology, UK). A 1000-ohm resistor was positioned between each anode–cathode electrode pair to enable voltage measurement.

In addition, a reference electrode (R218, PY Electrochemistry, China) was installed to measure half-cell potentials at the anode. Data acquisition was performed using software configured to log readings every 1 minute. The data was automatically stored on a cloud platform provided by the data logger manufacturer for remote access

and analysis. For operational consistency, the sewage in the circulation system was refreshed every 30 minutes to ensure the biosensor was continuously exposed to fresh raw sewage samples. The excess or overflowed sewage was directed back into the STP drainage system to maintain a closed-loop and environmentally compliant setup.

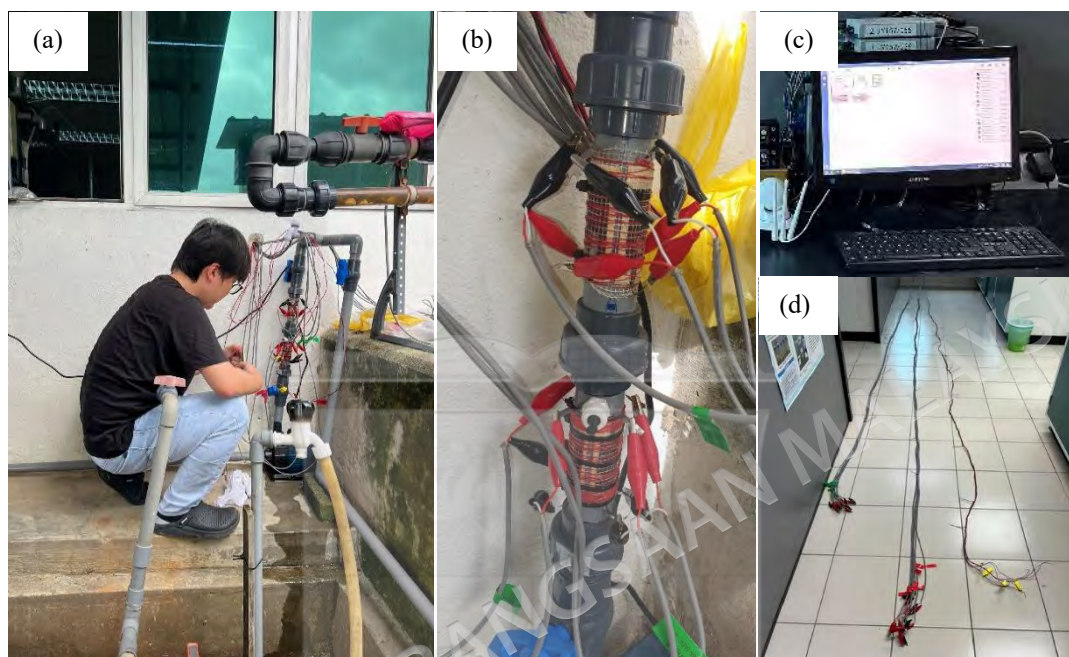


Figure 3.8 (a) On-site MFCSe installation, (b) Assembled MFCSe, (c) Data acquisition system, and (d) cable connection.

## **CHAPTER IV**

### **RESULT AND DISCUSSION**

#### **4.1 INTRODUCTION**

This chapter will be divided into four main sections to discuss about the results obtained from various analytical methods and experiments. The first and second sections will discuss the characteristics of the modified electrodes, the signal responses from the sensors during pollutant testing, and the correlation between signal data and COD results for both MESe and MFCSe, respectively. The signal responses and durability of both sensor types are compared at the end of the second section with a small conclusion highlighting on the superior sensor. In the third section, the sensor data obtained from the IoT platform and alarm notifications triggered during anomalies will be elaborated. Lastly, the performance and stability of MFCSe when deployed under actual municipal wastewater treatment conditions are evaluated.

#### **4.2 MICROBIAL ELECTROCHEMICAL SENSOR (MESE)**

##### **4.2.1 Physicochemical analysis**

###### **a. SEM analysis**

Figure 4.1 presents SEM micrographs of the modified electrodes, with the untreated SS304L electrode serving as the control. The control electrode was mechanically polished using sandpaper to eliminate surface impurities and achieve a defined roughness profile (Figure 4.1 (a)). In Figure 4.1 (b), the electrode surface was coated

with carbon powder, which aggregated into distinct granules to form a cohesive layer when 5% w/v Nafion solution was employed as the binder. Figure 4.1 (c) shows the formation of a dense iron oxide layer on the electrode surface, resulting from the flame oxidation treatment. Meanwhile, Figure 4.1 (d) shows the morphological effect of acid bleaching, where the surface layer appeared recessed and inwardly curved, indicating substantial material etching and removal. Among the three surface modified electrodes, significant changes were exhibited on the carbon powder and flame oxidised MESe electrodes compared to acid bleached electrode. Nevertheless, the uneven and arched surface observed on the acid bleached electrode was resulted from the strong acid etching process, consistent with findings reported in a previous study involving different grades of stainless steel (Li et al. 2012).

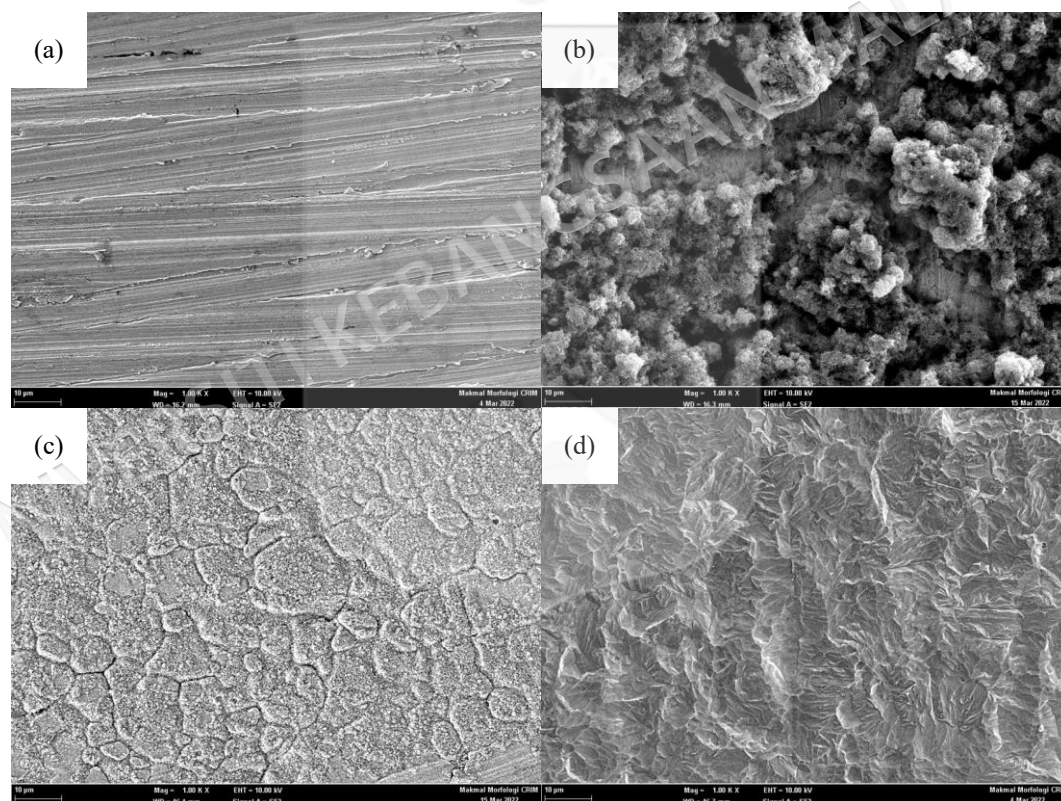


Figure 4.1 SEM images at 1000x magnification for (a) control, (b) carbon-coated, (c) flame oxidised, and (d) acid bleached MESe electrodes.

Among the three surface-modified electrodes, the carbon-coated electrode Figure 4.1 (b) exhibits the most favorable surface characteristics for biofilm attachment. The aggregated carbon granules create a highly rough and porous microstructure, which provides abundant anchoring sites for microbial cells and facilitates the retention of

EPS (Huang et al. 2023; Yang et al. 2025). Such a heterogeneous surface promotes rapid initial colonisation, supports the formation of thicker and more stable biofilms, and increases the effective electroactive surface area. Additionally, the carbon layer enhances surface conductivity and microbial–electrode contact (Mejía-López et al. 2023), both of which are beneficial for sustained electron transfer in microbial fuel cell systems. The flame oxidised electrode Figure 4.1 (c) also presents a surface morphology conducive to biofilm development, though to a lesser extent than the carbon-coated surface. The formation of a dense iron oxide layer introduces micro-scale texture and grain boundaries, which improve microbial adhesion compared to the untreated control (Yang et al. 2021). These surface features can enhance initial cell attachment and biofilm continuity by increasing surface energy and providing physical trapping sites (Yang et al. 2021). However, the relatively compact and less porous nature of the oxide layer may limit deep biofilm penetration and reduce long-term biofilm thickness compared to carbon-based coatings. In contrast, the acid bleached electrode Figure 4.1 (d) is expected to be the least favourable for biofilm attachment despite undergoing substantial surface modification. The strong acid etching process resulted in an irregular and inwardly curved surface, but the overall morphology appears comparatively smoother and less structurally heterogeneous than the carbon-coated and flame oxidised electrodes. The absence of an irregular surface reduces mechanical interlocking between microbial cells and the electrode surface, leading to weaker initial adhesion and increased susceptibility to biofilm detachment under operational shear forces (Yeo et al. 2023). Although acid bleaching may improve surface cleanliness and hydrophilicity, these effects alone are insufficient to compensate for the lack of physical anchoring features required for robust biofilm formation.

#### **b. EDX analysis**

Figure 4.2 shows the EDX elemental mapping for the modified MESe electrodes, while Table 4.1 shows the elemental composition present on each sample. Carbon is widely recognized as a biocompatible material, including in applications involving microbial fuel cells (Fan et al. 2021; Zhu et al. 2023). The carbon coating applied to the electrode served to significantly reduce iron (Fe) content on the surface from 60.7% to 9.4%, while simultaneously increasing the carbon (C) composition from 12.2% to 78.6%.

Notably, oxygen (O) was not detected in this sample. The coating also masked other metal elements such as manganese (Mn) and nickel (Ni), with Mn becoming undetectable and Ni reduced to 1.0%. This indicates that substantial and uniform coverage of the carbon powder was achieved during the modification process. In contrast, the flame oxidised electrode showed an increase in oxygen content from 3.2% (based on control) to 20.5%, consistent with previous findings that report the formation of a passive iron oxide layer during heat treatment. This oxide layer is known not only for its corrosion resistance but also for enhancing microbial adhesion due to its biocompatibility as well (Emerson. 2018).

Another study also found that the formation of a thick iron oxide layer during flame oxidation enhanced biofilm development at the anode, which enhanced the sensor's performance (Guo et al. 2014). However, the flame treatment also eliminated the chromium (Cr)-rich oxide layer originally present on the stainless steel, which serves as a protective barrier against corrosion. As a result, the flame oxidised electrode became more prone to rusting. Meanwhile, the C percentage on the flame-treated electrode decreased from 12.2% to 4.1%. For the acid bleached sample, the original iron oxide layer was completely removed, as indicated by the absence of O element in the EDX analysis. The remaining elemental composition was similar to that of the untreated control.

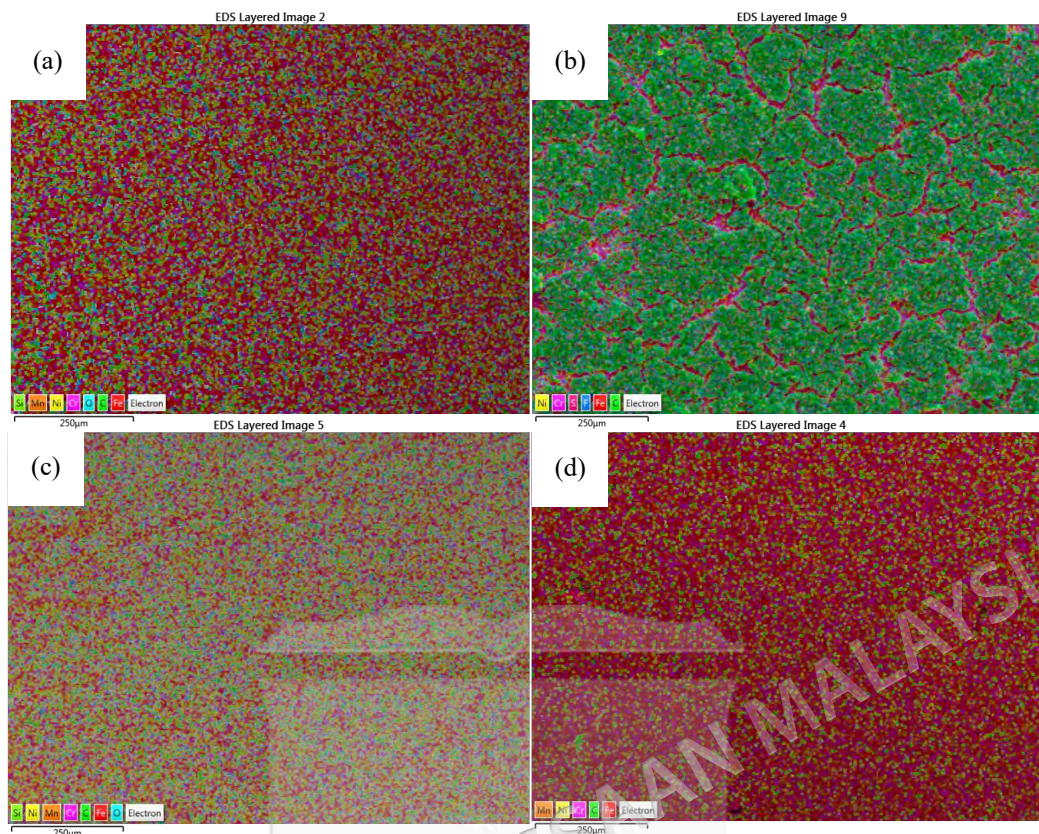


Figure 4.2 EDX elemental mapping at 500x magnification for (a) control, (b) carbon-coated, (c) flame oxidised, and (d) acid bleached MESE electrodes.

Table 4.1 EDX values of the modified MESE electrodes.

| Element (%) | Control         | Carbon-coated   | Flame oxidised  | Acid bleached   |
|-------------|-----------------|-----------------|-----------------|-----------------|
| <b>Fe</b>   | $60.7 \pm 0.4$  | $9.4 \pm 0.1$   | $57.1 \pm 0.2$  | $65.2 \pm 0.3$  |
| <b>C</b>    | $12.2 \pm 0.3$  | $78.6 \pm 0.1$  | $4.1 \pm 0.1$   | $8.1 \pm 0.2$   |
| <b>O</b>    | $3.2 \pm 0.1$   | N.D.            | $20.5 \pm 0.1$  | N.D.            |
| <b>Cr</b>   | $15.6 \pm 0.2$  | $2.6 \pm 0.1$   | $13.2 \pm 0.1$  | $17.4 \pm 0.2$  |
| <b>Ni</b>   | $6.8 \pm 0.3$   | $1.0 \pm 0.1$   | $3.9 \pm 0.1$   | $7.6 \pm 0.2$   |
| <b>Mn</b>   | $1.1 \pm 0.2$   | -               | $0.9 \pm 0.1$   | $1.3 \pm 0.2$   |
| <b>Si</b>   | $0.4 \pm 0.0$   | -               | $0.3 \pm 0.0$   | $0.4 \pm 0.0$   |
| <b>F</b>    | -               | $7.8 \pm 0.1$   | -               | -               |
| <b>S</b>    | -               | $0.7 \pm 0.0$   | -               | -               |
| <b>Sum</b>  | $100.0 \pm 0.4$ | $100.1 \pm 0.4$ | $100.0 \pm 0.2$ | $100.0 \pm 0.3$ |

### c. AFM analysis

Figure 4.3 depicts the topography imaging, and Table 4.2 shows the line profiling values of the modified samples. Based on the Figure 4.3 (a) and Figure 4.3 (d), the topography of the control and flame oxidised samples are almost similar. The histograms of both samples show that the peak distribution value occurred between 10 and 11 nm. However, the flame oxidised sample (Figure 4.3 (c)) skewed to the left more than the control ( $R_{sk}$  value 0.114 vs. -0.23) due to the thickening of the iron oxide layer on the sample's surface. Apart from the  $R_{sk}$  value, there are no other significant differences in the line profiling results (min, max, mid, mean  $\leq \pm 5\%$ ;  $R_{pv}$ ,  $R_q$ ,  $R_a$ ,  $R_z$ ,  $R_{ku} \leq \pm 35\%$ ). Meanwhile, the comparison between the carbon-coated (Figure 4.3 (b)) and acid bleached samples to the control showed noticeable roughness distribution (min, max, mid, mean between -100 ~ +5%). Further analysis of the line profiling showed higher differentials in  $R_{pv}$ ,  $R_q$ ,  $R_a$ ,  $R_z$ ,  $R_{sk}$ , and  $R_{ku}$  values (+20 ~ +1550%). One of the noticeable high differentials is the values between peaks and valleys ( $R_{pv}$ ,  $R_z$ ). This is because a layer of carbon coating was added to the sample surface. The layer was broken up due to the drying process, causing depth valleys. Meanwhile, a layer of SS was removed from the surface, also causing the large peaks and valleys to be different. The rough surface also caused the largest spikiness ( $R_{ku}$ ) among the samples, with the highest value recorded as 40.832. Topography structure and surface roughness were believed to facilitate bacterial attachment at the beginning, shorten the time of enrichment, and finally increase detection (Champigneux et al. 2019; González-Martínez et al. 2023). However, this is not the case when compared to coating materials and surface electrochemistry. The surface properties and characteristics dictate the biosensor performance, which in turn is dominated by the attached functional microorganisms that possess certain reaction and metabolism capabilities. Similar effects were also observed by Pierra et al., where the increasing roughness on the tested electrodes did not significantly impact the bacterial attachment or current generation (Pierra et al. 2018).

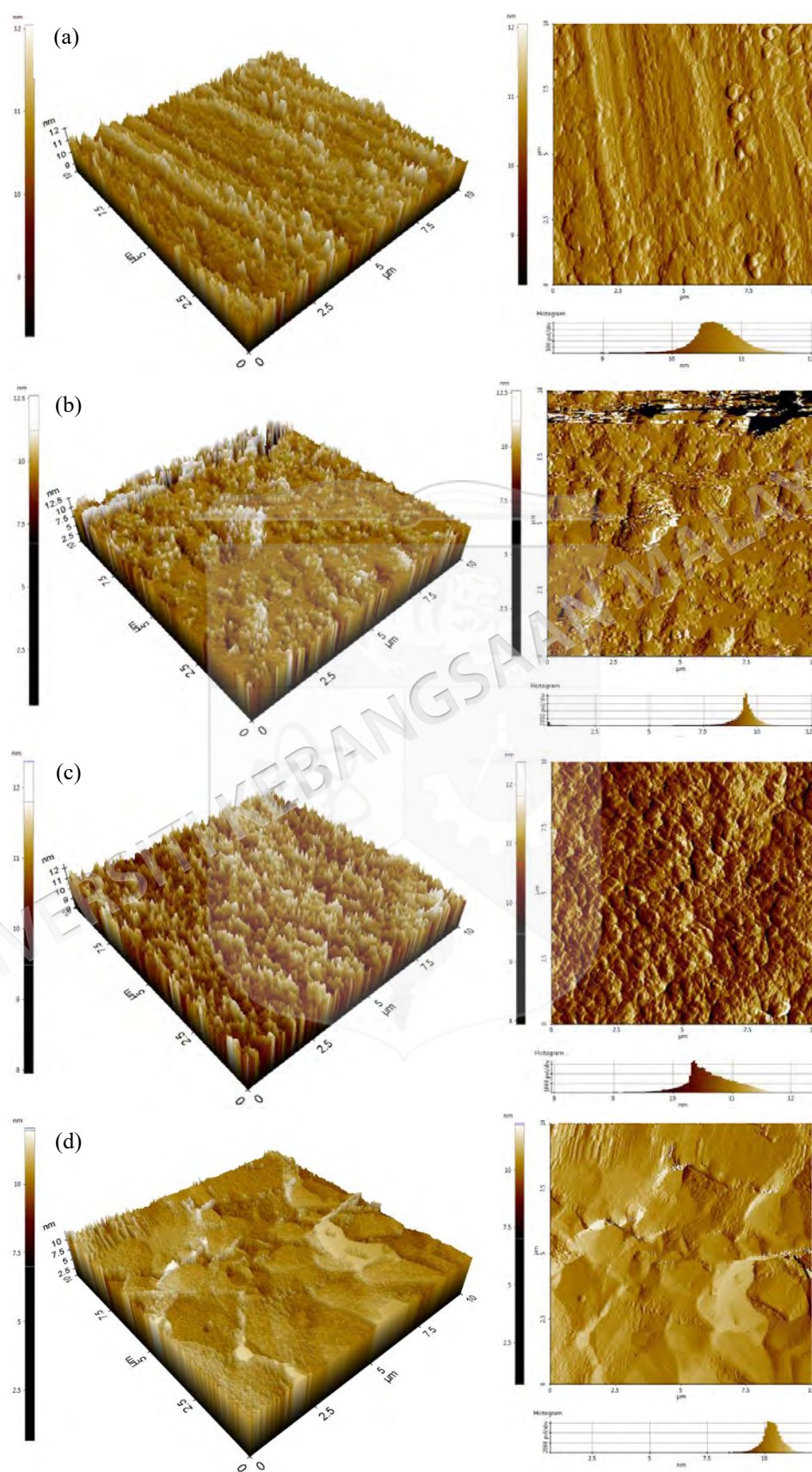


Figure 4.3

Topography imaging of the electrode surfaces (left images are 3D; right images are 2D & histogram): (a) Control, (b) carbon powder coated, (c) flame oxidised, and (d) acid bleached.

Table 4.2 AFM line profiling parameters.

| Sample*                     | Min   | Max    | Mid    | Mean   | R <sub>pv</sub> | R <sub>q</sub> | R <sub>a</sub> | R <sub>z</sub> | R <sub>sk</sub> | R <sub>ku</sub> |
|-----------------------------|-------|--------|--------|--------|-----------------|----------------|----------------|----------------|-----------------|-----------------|
| Control                     | 8.282 | 12.049 | 10.166 | 10.644 | 3.766           | 0.355          | 0.264          | 3.720          | -0.230          | 5.589           |
| Carbon powder <sup>1</sup>  | 0.291 | 12.600 | 6.445  | 9.141  | 12.309          | 1.720          | 0.844          | 12.265         | -3.793          | 18.990          |
| Flame oxidised <sup>2</sup> | 7.948 | 12.380 | 10.164 | 10.636 | 4.432           | 0.410          | 0.323          | 4.044          | 0.114           | 3.614           |
| Acid bleach <sup>3</sup>    | 0.673 | 12.036 | 6.354  | 10.296 | 11.363          | 0.487          | 0.336          | 11.030         | -2.618          | 40.832          |

Note:

<sup>1</sup> Carbon powder spray-coated breaking up in small pieces contributes to the roughness and highest differential between peaks and valleys (R<sub>pv</sub>).

<sup>2</sup> Positive skewness (R<sub>sk</sub>) with less spikiness (R<sub>ku</sub>) indicated the passivation layer due to flame oxidation.

<sup>3</sup> A layer of the metal surface was removed in bleach, causing a larger differential between peaks and valleys (R<sub>pv</sub>). (R<sub>q</sub> represents root-mean squared roughness, R<sub>a</sub> represents average roughness, and R<sub>z</sub> represents the ten point average roughness)

#### d. RAMAN analysis

Figure 4.4 presents the Raman spectra of SS304L electrodes subjected to different surface treatments, highlighting the distinct chemical signatures induced by each modification. The control electrode (Figure 4.4 (a)) exhibited a broad, low-intensity hump with no well-defined peaks, suggesting a lack of Raman-active surface structures on the untreated stainless steel. In contrast, the carbon-coated electrode (Figure 4.4 (b)) showed two prominent peaks at 1355 cm<sup>-1</sup> (disorganized-band) and 1593 cm<sup>-1</sup> (graphite-band) (Dubale et al. 2014; Kaneki et al. 2024), corresponding to disordered and graphitic sp<sup>2</sup>-hybridized carbon structures, respectively. These features confirm the successful deposition of amorphous carbon, which enhances microbial adhesion and electron transfer, as also reported in recent studies on carbon-modified electrodes for bioelectrochemical applications (Cheng et al. 2022; Jukanti et al. 2026). The flame oxidised electrode (Figure 4.4 (c)) revealed a distinct peak at 706 cm<sup>-1</sup> (Zhao et al. 2015), characteristic of magnetite (Fe<sub>3</sub>O<sub>4</sub>) confirming the formation of an iron oxide layer as a result of thermal oxidation. This surface oxidation is known to improve surface roughness, hydrophilicity, and biofilm formation, thereby enhancing current output in microbial electrochemical systems (Guo et al. 2014). However, it is important to note that while such oxidation boosts performance, it may also increase susceptibility to corrosion if not stabilized (Ledezma et al. 2015). The acid bleached electrode (Figure 4.4 (d)), in contrast, displayed no discernible peaks across the Raman shift range, indicating the complete removal of polarizable surface oxides and organics by the

aggressive acid treatment. This absence of Raman-active species suggests a chemically inert surface, consistent with prior findings that show acid etching can inhibit microbial attachment due to loss of bioactive functional groups (Ban et al. 2020). Raman spectroscopy relies on vibrational changes in molecular polarizability, which provides valuable insight into surface functionality critical to biosensor performance. These spectra clearly demonstrate how each surface treatment affects the electrode's chemical composition, ultimately influencing microbial colonization and electron transfer in bioelectrochemical systems.

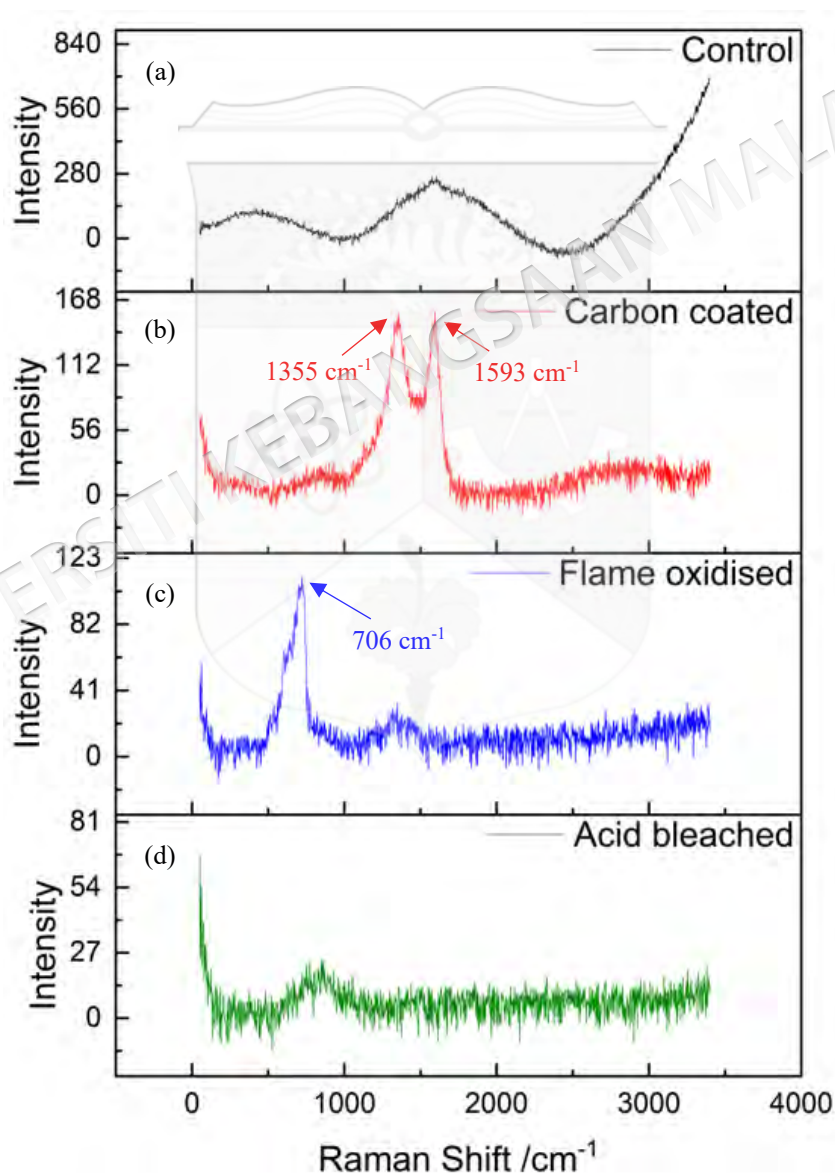


Figure 4.4

Raman spectrogram of (a) control, (b) carbon-coated, (c) flame oxidised, and (d) acid bleached MESe electrodes.

#### 4.2.2 Electrochemical properties

Figure 4.5 and Figure 4.6 presents the electrochemical characterization of biosensors assembled using different surface-modified SS304L electrodes. These evaluations were conducted through electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) after the biosensors had undergone sufficient microbial enrichment to achieve a stable and reproducible current output.

##### a. EIS analysis

Figure 4.5 shows the EIS results for both the biosensor (whole-cell) and the isolated anode compartments (half-cell), which allows a deeper interpretation of the internal resistance across the electrode configurations. From the whole-cell EIS analysis, it was observed that the control and acid bleached samples exhibited the highest internal resistance, ranging between 1600 and 1700  $\Omega$ . This high resistance is primarily due to elevated polarization resistance ( $R_p$ ) recorded at approximately 1500 to 1600  $\Omega$ , indicating poor electron transfer capabilities and limited microbial activity. In contrast, the electrodes modified with carbon coating and flame oxidised demonstrated a substantial reduction in  $R_p$  values, which were 286 and 189  $\Omega$ , respectively. This suggested that the surface modifications significantly enhanced the electrode's electrochemical activity, likely due to improved microbial adhesion, better electron transfer pathways, and increased biocompatibility. Interestingly, the solution resistance ( $R_s$ ) remained fairly consistent across all samples, with values between 90 and 97  $\Omega$ , implying that the variations in total resistance were primarily driven by interfacial properties at the electrode surfaces rather than the electrolyte medium.

To further distinguish the contributions of the anode surfaces, the anode-specific EIS data were analyzed independently by eliminating the influence of the cathode (as shown in the inserted figure). The  $R_p$  values of the anodes followed the same trend as the whole-cell measurements: acid bleached > control > carbon-coated > flame oxidised. This order reflected the degree of microbial colonization and activity supported by each surface. Acid bleached electrodes lacked surface oxides and biocompatible functional groups, which limited the microbial attachment and electron transfer, while flame

oxidised surface was enriched with iron oxides that provided a more favorable interface for microbial electron transport. Additionally, the calculated polarization resistance at the cathode showed clear dependence on the anode performance. The cathodic  $R_p$  values were estimated at 1186  $\Omega$  (control), 102  $\Omega$  (carbon-coated), 154  $\Omega$  (flame oxidised), and 975  $\Omega$  (acid bleached). These values demonstrate that anode modifications not only influence the local bioelectrochemical environment but also affect the overall system behavior, including cathodic kinetics. This phenomenon could be attributed to potential shifts within the system and changes in microbial community structure, as electron flow and metabolic interactions at the anode directly affect redox balance at the cathode.

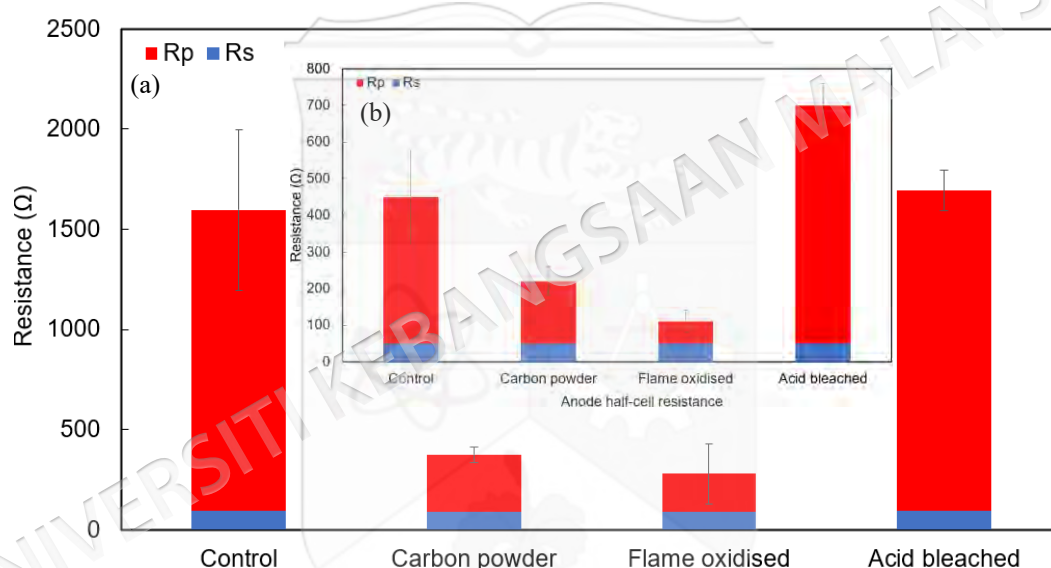


Figure 4.5 (a) Cell and (b) anode resistances of MESe with modified electrodes, where  $R_p$  and  $R_s$  represent polarization and solution resistances, respectively.

The enhanced electrochemical activity inferred from EIS measurements can be partially explained by the surface modifications observed in the SEM and AFM analyses; however, the improvement cannot be attributed to surface roughness alone. SEM images reveal that the carbon-coated and flame-oxidised electrodes possess distinct surface morphologies that promote microbial attachment through increased surface heterogeneity and microbial anchoring sites (Liu et al. 2023; Rehman et al. 2025). In particular, the carbon-coated electrode exhibits a highly porous and granular structure, which not only facilitates biofilm growth (Yang et al. 2020), but also improves electrical

connectivity between microorganisms and the electrode surface (Agrahari et al. 2022). This morphology is consistent with lower charge transfer resistance observed in EIS, as intimate microbe–electrode contact and conductive pathways enhance electron transport. AFM analysis further shows that although both carbon-coated and acid bleached electrodes exhibit significantly higher roughness parameters ( $R_a$ ,  $R_q$ ,  $R_z$ ,  $R_{pv}$ ) compared to the control, increased roughness does not uniformly translate into improved bioelectrochemical performance. The acid bleached electrode, despite displaying large peak-to-valley differences and high spikiness ( $R^{ku}$ ), presents a recessed and inwardly curved surface that lacks the structural heterogeneity and chemical functionality required for stable biofilm development. This supports the observation that electrochemical enhancement detected by EIS is more strongly governed by surface chemistry, conductivity, and biocompatibility than by roughness magnitude alone.

Collectively, the SEM and AFM results suggest that surface modifications enhance electrochemical activity primarily when roughness is coupled with favourable surface composition and electron transfer pathways (Mu et al. 2023; Ribeiro et al. 2023). Carbon coating and flame oxidation introduce surface features and chemical states that support microbial adhesion and extracellular electron transfer, thereby reducing interfacial resistance and improving EIS response. In contrast, excessive or poorly structured roughness, as observed in the acid bleached electrode, does not necessarily improve microbial attachment or electrochemical performance. These findings support the EIS interpretation that surface modification enhances electrode activity through synergistic effects of microbial compatibility, conductive interfaces, and effective electron transfer rather than roughness alone.

Overall, these findings highlight the crucial role of surface modification in enhancing the electrochemical performance of microbial electrochemical sensors. Lower internal resistance at the anode not only facilitates efficient electron transfer but also contributes to more effective biofilm development and system stability. The strong correlation between anode performance and cathodic behavior supports previous findings that emphasize the importance of electrode interface design in bioelectrochemical systems (Buitrón et al. 2017; Fischer et al. 2022).

## b. CV Analysis

Figure 4.6 presents the cyclic voltammograms recorded from the MESe electrodes with different surface modifications and reveals a clear dependence of electrochemical behavior on electrode surface characteristics. Among the tested sensors, the electrochemical activity followed the order carbon-coated > flame oxidised > control > acid bleached, as reflected by their respective current densities. The carbon-coated MESe exhibited the highest electrochemical activity, reaching a maximum current density plateau ( $J_{\max}$ ) of approximately  $1.8 \text{ A m}^{-2}$  at around  $-0.2 \text{ V vs. SHE}$ . This potential corresponds to the typical operating range for bioelectrochemical biosensors and indicates efficient bioelectrochemical oxidation and electron transfer at the electrode–biofilm interface (Yamashita et al. 2016).

The flame oxidised MESe demonstrated moderate electrochemical performance, achieving a maximum current density of approximately  $0.8 \text{ A m}^{-2}$ . Its cyclic voltammogram displayed two distinct oxidation features: a primary response occurring at  $-0.2 \text{ V vs. SHE}$ , similar to the carbon-coated electrode, and a secondary oxidation peak at approximately  $+0.5 \text{ V vs. SHE}$ . The latter is attributed to water hydrolysis facilitated by the magnetite layer formed during the flame oxidation process, consistent with previous reports on iron oxide–modified electrodes (Halley et al. 2012). While flame oxidation enhanced electrochemical activity relative to the untreated electrode, its current response remained lower than that of the carbon-coated MESe, suggesting limitations associated with the compact nature of the oxide layer and comparatively lower conductivity.

In contrast, the control and acid bleached MESe produced relatively flat CV profiles, indicating minimal redox activity. The control electrode exhibited a slightly higher current response, with maximum values of approximately  $1.0 \text{ A m}^{-2}$ , compared to the acid bleached electrode, whose current densities remained below  $0.5 \text{ A m}^{-2}$ . Notably, the acid bleached MESe showed the poorest electrochemical performance, highlighting its unsuitability for supporting electroactive microbial growth. This observation is consistent with previous studies reporting that aggressive acid treatments, such as aqua regia etching, can hinder microbial adhesion and biofilm formation on

stainless steel surfaces (Ban et al. 2020). The severely etched and recessed surface produced by acid bleaching likely impaired stable biofilm development, resulting in the negligible current response observed in the CV measurements.

These electrochemical trends are in strong agreement with the surface morphology and topographical features observed in the SEM and AFM analyses. The superior performance of the carbon-coated MESe can be attributed to its highly rough, porous, and heterogeneous surface, which provided abundant anchoring sites for microbial attachment, enhanced retention of extracellular polymeric substances, and improved microbial-electrode contact. AFM results further confirmed pronounced peak-valley structures that increased the effective electroactive surface area without compromising surface connectivity. Similarly, the flame oxidised electrode benefited from micro-scale texturing and the formation of an iron oxide layer, which improved microbial adhesion relative to the control electrode. Conversely, despite exhibiting elevated roughness parameters in AFM analysis, the acid bleached electrode displayed an irregular yet inwardly curved morphology under SEM, lacking well-defined micro- or nano-scale features necessary for effective microbial anchoring. This mismatch between surface roughness magnitude and functional surface structure explains the poor electrochemical response observed in the CV measurements and reinforces the conclusion that electrochemical performance in MESe electrodes is governed not solely by roughness, but by the combined influence of surface morphology, chemical composition, conductivity, and biocompatibility.

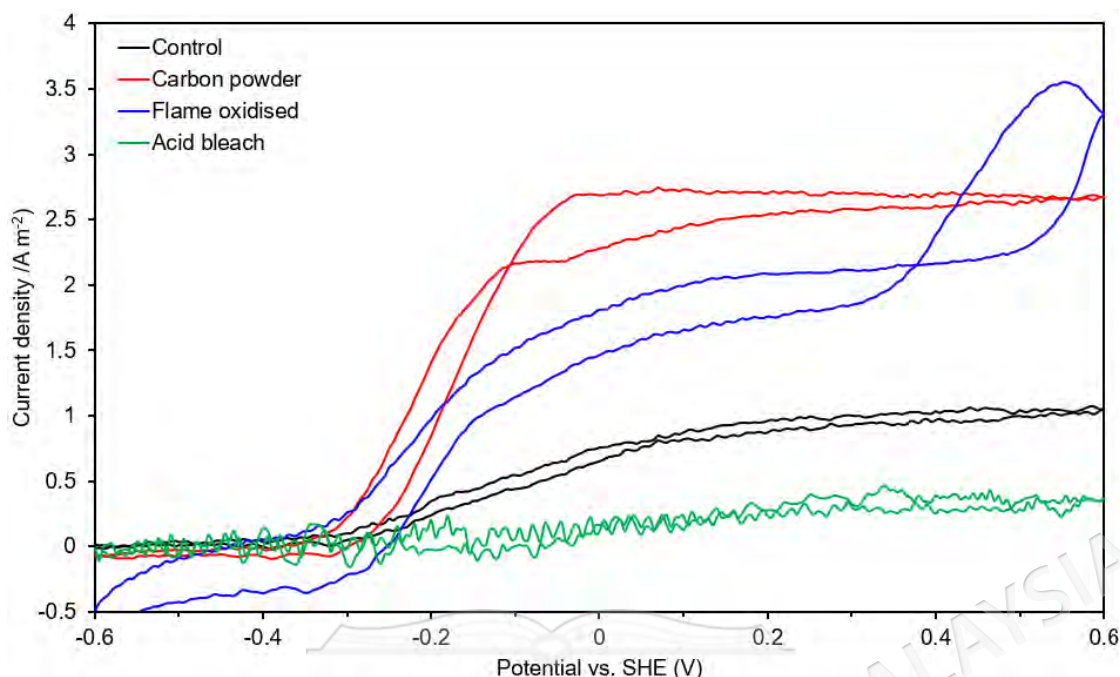


Figure 4.6 Cyclic voltammogram of MESe with modified electrodes.

Based on Figure 4.6, the distinct plateau current densities observed for the four MESe electrodes arise from differences in the rate-limiting processes governing bioelectrochemical oxidation. In bioelectrochemical sensors, the current plateau reflects a condition in which further increases in applied potential no longer enhance the reaction rate (Molderez et al. 2021), and the current becomes limited by microbial metabolic activity and charge transfer at the biofilm–electrode interface. The carbon-coated electrode exhibits the highest plateau current due to its highly porous and conductive surface, which supports stable biofilm formation and provides efficient electron transfer pathways (Santoro et al. 2014; Senthilkumar et al. 2023). The flame oxidised electrode reaches an intermediate plateau current, as surface oxidation introduces micro-scale texture and improves microbial adhesion relative to the control; however, the presence of a compact iron oxide layer imposes additional resistance to electron transport (Huang et al. 2016), limiting the maximum achievable current despite enhanced surface reactivity. The control electrode displays a lower plateau current because its comparatively smooth surface restricts microbial coverage and reduces the number of active electron transfer sites, resulting in a modest limiting current once microbial oxidation becomes rate-determining. In contrast, the acid bleached electrode shows the lowest plateau current, indicating poor bioelectrochemical performance; although AFM analysis revealed increased roughness and the SEM observations

showed an arched and inwardly curved morphology that hindered biofilm attachment. Consequently, biofilm formation becomes the dominant limiting factor, leading to a substantially reduced current response. Overall, the variation in plateau currents demonstrates that the maximum electrochemical output of MESe electrodes is governed not solely by applied potential, but by the combined effects of surface roughness, morphology, and material biocompatibility.

#### 4.2.3 Pollutants testing

This section presents the signal responses of MESes incorporating various surface-modified SS304L electrodes (control, carbon-coated, flame oxidised, acid bleached). These biosensors were tested under exposure to four model pollutants, which were sodium acetate, ethanol, palm oil mill effluent (POME), and thinner to assess their real-time signal responsiveness and sensitivity. The chemical oxygen demand (COD), pH, temperature, and conductivity of the wastewater was measured and recorded as well.

##### a. Sodium acetate

Sodium acetate was chosen as one of the model pollutants because it served as the enrichment medium during biofilm formation, making it more readily identifiable by the MESes, as demonstrated by the distinct spike signals observed (Figure 4.7). During the sodium acetate pollutant test, a well-defined step-wise increase in current density was observed for both the carbon-coated and flame oxidised electrodes, corresponding closely with each acetate injection from 1<sup>st</sup> to the 8<sup>th</sup> hour. MESe with the flame oxidised electrode reached a peak current density of up to  $1.13 \text{ A m}^{-2}$  during the entire pollutant testing period, outperforming the control MESe ( $0.33 \text{ A m}^{-2}$ ), carbon-coated MESe ( $1.09 \text{ A m}^{-2}$ ), and acid bleached MESe ( $0.24 \text{ A m}^{-2}$ ). Similarly, carbon-coated MESe also performed well due to its high carbon content (78.6%), which possessed excellent biocompatibility to promote microbial growth and efficient charge transfer. In stark contrast, the unmodified control MESe and acid bleached MESe exhibited negligible current changes throughout the acetate test. This suggests poor microbial colonization and weak electron transfer capabilities, likely due to their lower surface energy and absence of catalytic or adhesion-promoting functional groups (Ojha et al. 2025;

Perchikov et al. 2024). These findings underscore the critical importance of surface chemistry and morphology in shaping biofilm-electrode interaction dynamics.

In addition to the electrochemical response, the COD profile recorded during the sodium acetate test provides important insight into the relationship between organic substrate availability and current generation. As shown in Figure 4.7, increases in COD following acetate addition were accompanied by clear step-wise rises in current density for the carbon-coated and flame oxidised MESes, indicating effective microbial oxidation of acetate and conversion of biodegradable organic matter into electrical output. The subsequent stabilisation or gradual reduction in COD reflects progressive substrate consumption by the electroactive biofilm and is consistent with the sustained current levels observed once a stable metabolic state was achieved. In contrast, the control and acid bleached MESes exhibited minimal changes in current density despite the presence of measurable COD, demonstrating that organic loading alone is insufficient to produce a strong electrochemical signal when biofilm formation and electron transfer are limited (Greenman et al. 2021). This coupling between COD variation and current response observed for the well-performing electrodes highlights the ability of MESes to reflect changes in biodegradable organic content. With appropriate calibration and further validation under real wastewater conditions, such COD-responsive behavior has strong potential to be extended toward real-time BOD sensing (Yamei Ma et al. 2022). This offers a faster and continuous alternative to conventional laboratory-based BOD measurements.

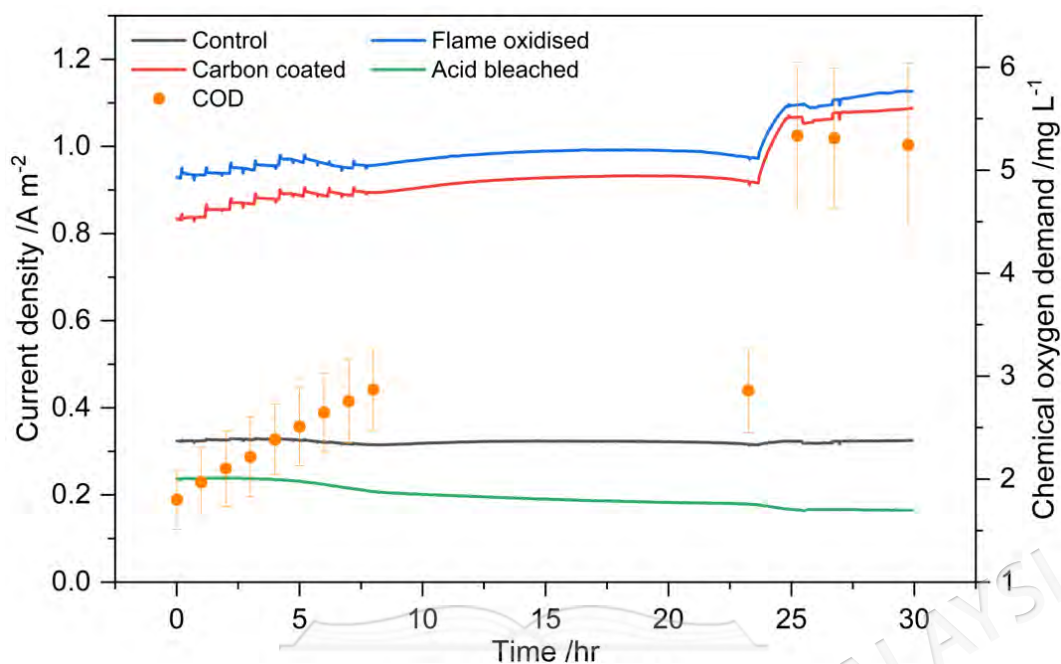


Figure 4.7 Signal profile and COD during pollutants testing for sodium acetate.

#### b. Ethanol

During the ethanol pollutant test, a notable deviation from the other tests was observed, characterized by two distinct drops in current density. The initial current density decline was attributed to ethanol hydration, as the wastewater contained a high ethanol concentration of nearly  $40,000 \text{ mg L}^{-1}$ . The second current density drop occurred after dilution was due to the increased atmospheric exposure when the tank was partially emptied and then refilled with tap water (Figure 4.8), which caused aeration within the tank. The anode chamber experienced increased exposure to ambient air, leading to a sudden rise in oxygen availability and changes in headspace pressure. Oxygen intrusion into the anode environment disrupts the strictly anaerobic conditions required for electroactive microorganisms (Lu et al. 2021), as oxygen acts as a competing terminal electron acceptor (Wei et al. 2024). As a result, microorganisms preferentially divert electrons toward oxygen reduction rather than transferring them to the anode, causing an immediate decline in current density (Quan et al. 2012). Interestingly, the control sensor showed a delayed signal fluctuation during the dumping and dilution phases, which was due to the reflecting transient abiotic electrochemical effects or minimal microbial stress adaptation. The acid bleached sensor again remained essentially flat, confirming its ineffectiveness across various conditions.

Based on Figure 4.8, periods of declining current density coincided with increasing COD levels during the incremental test. This phenomenon indicated that although organic matter was present in high concentration, it was not effectively metabolised by the electroactive microorganisms. This behavior can be attributed to the toxic effect of ethanol at elevated concentrations (Horinouchi et al. 2018), which is known to damage microbial cell membranes and disrupt enzymatic activity of the microbes (Cao et al. 2017). As microbial activity declined, substrate utilisation was inhibited, leading to COD accumulation while electrical output decreased. Following dilution, partial recovery or further instability in current response reflected the combined effects of reduced ethanol concentration and environmental disturbance. In short, these observations demonstrate that high COD does not necessarily translate into higher current generation in MESEs when microbial viability is compromised, highlighting the sensitivity of bioelectrochemical sensors to toxic organic compounds such as ethanol.

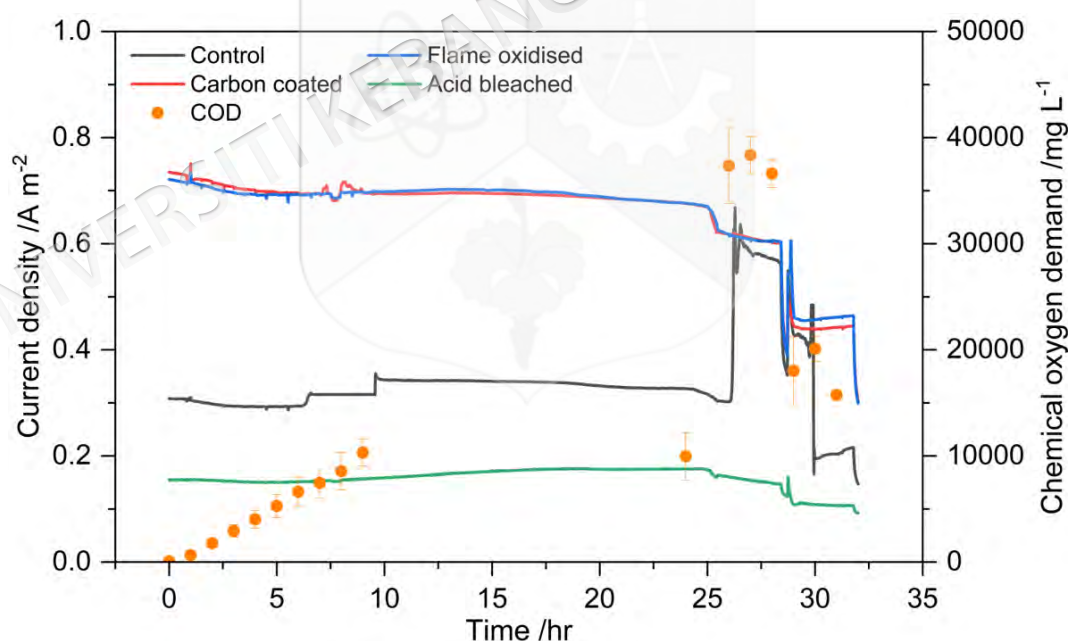


Figure 4.8 Signal profile and COD during pollutants testing for ethanol.

### c. Palm oil mill effluent (POME)

In the POME test, the response patterns were more subdued and temporally shifted. The carbon-coated sensor exhibited a delayed but significant increase in current density (up to  $1.8 \text{ A m}^{-2}$ ) several hours after the dumping event. This reflects the time required for the microbial community to adapt and metabolize the complex, slowly degradable organics present in POME. Flame oxidised sensors showed similar but less pronounced behavior. Notably, the flatter and slower signal response contrasts with the sharp peaks seen in the acetate test, highlighting the sensor's sensitivity to substrate complexity and biodegradability. COD levels remained consistently high, indicating persistent organic loading. The control and acid bleached electrodes again failed to show meaningful signal variation, rendering them unsuitable for complex wastewater monitoring (Figure 4.9).

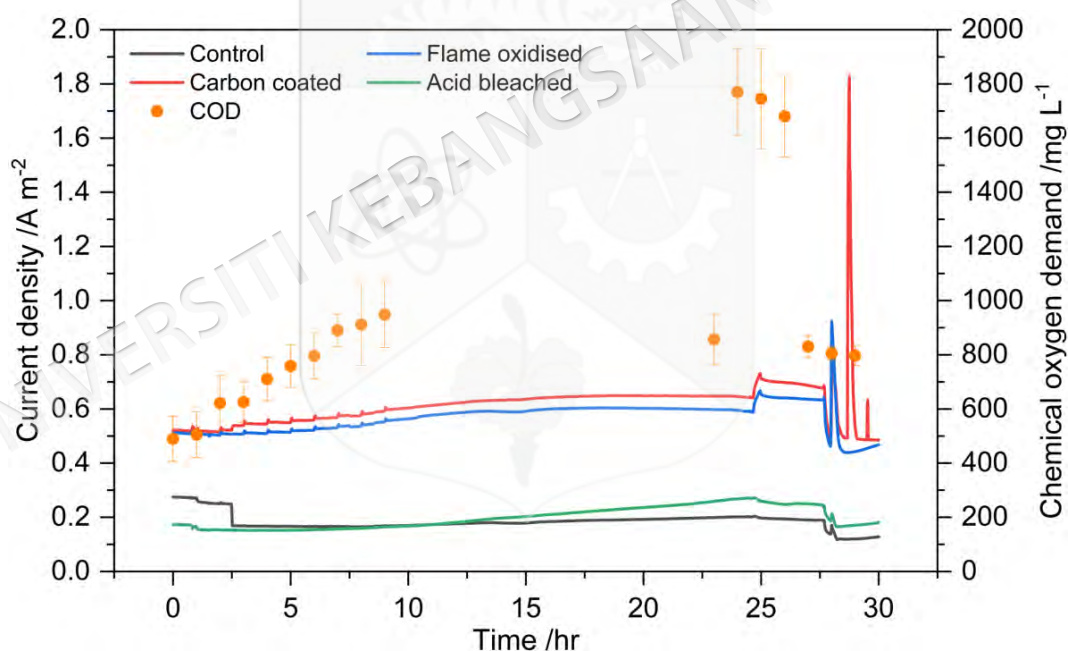


Figure 4.9 Signal profile and COD during pollutants testing for POME.

The COD levels remained consistently high throughout the experiment, reflecting the complex composition of POME, which consists of a heterogeneous mixture of carbohydrates, proteins, lipids, and other slowly degradable organic compounds (Ho et al. 2025; Samavati et al. 2025). Unlike sodium acetate, where increases in COD were rapidly translated into higher current densities through

immediate microbial oxidation, the elevated COD associated with POME did not result in an immediate electrochemical response. Instead, current generation increased only after an extended acclimation period, indicating that the electroactive microbial community required time to adapt and enzymatically process the diverse and complex substrates present. This delayed coupling between COD and current output contrasts sharply with the ethanol test, where high COD coincided with suppressed current due to microbial inhibition. In the case of POME, high COD persisted without inducing acute toxicity. However, its limited biodegradability and complex organic matrix constrained the rate of electron generation, leading to a flatter and slower step-wise signal response. These observations demonstrate that while COD reflects total organic load, the bioelectrochemical response of MESes is strongly influenced by substrate composition and biodegradability, explaining the slower and more subdued current profiles observed for POME when compared to readily degradable or inhibitory organic pollutants such as sodium acetate.

#### **d. Thinner**

As shown in Figure 4.10, each incremental addition of thinner resulted in a pronounced decrease in current density, producing troughs rather than peaks in the electrochemical signal. This response contrasts sharply with the behaviour observed for biodegradable substrates such as sodium acetate and highlights the inhibitory nature of thinner on electroactive microbial activity. Despite measurable increases in COD following thinner addition, the current density consistently declined, indicating that the organic content introduced by thinner was not bioavailable for microbial oxidation. Instead, the organic solvents present in thinner mostly comprised of toluene exerted a toxic effect on the biofilm (González-Cortés et al. 2024), suppressing microbial metabolism and extracellular electron transfer. As a result, higher COD levels coincided with lower current output, demonstrating a clear decoupling between organic loading and electrochemical response.

This inhibitory behavior is consistent with previous studies involving highly toxic organic compounds. For example, previous work reported that successive injections of formaldehyde at concentrations of 0.25%, 0.5%, and 1.0% caused

progressive decreases in voltage output in microbial fuel cells, with the most severe suppression observed at the highest concentration (Chu et al. 2021). Formaldehyde and thinner share comparable toxicity profiles, as both contain reactive organic solvents capable of disrupting microbial cell membranes, denaturing redox-active proteins, and impairing enzymatic pathways essential for electron transfer. In the present study, the repeated troughs in current density following thinner addition suggest cumulative microbial inhibition rather than transient metabolic adaptation.

Compared to ethanol, which exhibited a temporary suppression followed by partial recovery upon dilution, the response to thinner showed little evidence of biofilm resilience or adaptation. Similarly, unlike POME, where high COD persisted due to complex but slowly biodegradable compounds, thinner introduced readily detectable organic content that was fundamentally non-biodegradable and toxic to electroactive microorganisms (Al-Hameedi et al. 2020). The control and acid bleached MESes were particularly susceptible to this inhibition, showing sustained low current output throughout the test. Overall, the inverse relationship between COD and current density observed during thinner exposure confirms that MES response is governed not only by organic concentration but also by substrate toxicity and microbial compatibility, reinforcing the capability of MESes to distinguish inhibitory pollutants from biodegradable organic loads.

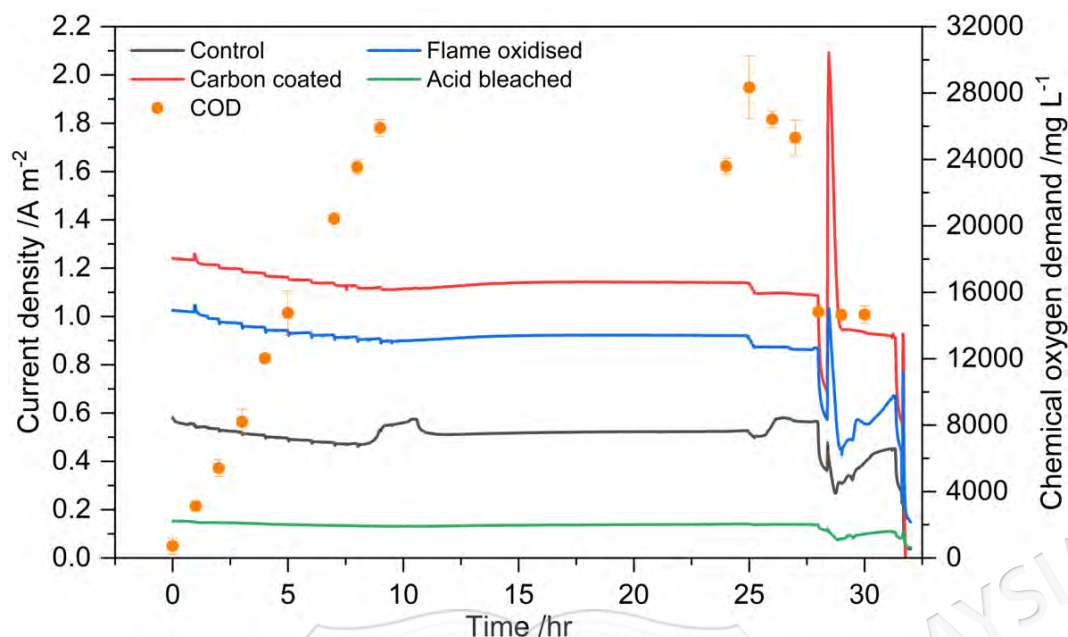


Figure 4.10 Signal profile and COD during pollutants testing for thinner.

The results clearly affirm that both carbon-coated and flame oxidised electrodes significantly enhance sensor responsiveness, attributed to improved biocompatibility, surface roughness, and chemical functionality. Meanwhile, unmodified and acid bleached surfaces suffer from poor electron transfer, low microbial affinity, and lack of catalytic activity. These findings provide compelling evidence that proper surface modification is essential for reliable MESe performance. Carbon-coated SS304L emerged as the superior configuration due to its strong biofilm compatibility and high current output across all pollutant types, including toxic and complex organic matrices. The flame oxidised electrode is potentially limited by corrosion risks and less stable surface composition over long-term exposure. Acid-bleaching may be counterproductive for BES-based sensors as it removes essential metal oxides and reduces microbial affinity. The control electrode's minimal performance further supports the necessity of functional surface engineering. From an application standpoint, these results suggest that carbon-modified stainless steel electrodes can offer a low-cost, scalable platform for real-time wastewater quality monitoring.

#### 4.2.4 Correlation data

Figure 4.11 presents the linear correlation between current density and chemical oxygen demand (COD) for microbial electrochemical sensors (MESes) using different surface-modified SS304L electrodes. The relationship between these two parameters is essential to evaluate the reliability and sensitivity of the MES system as an alternative to conventional COD-based water quality monitoring.

Among the four pollutant scenarios, the strongest correlation was observed in the thinner test (Figure 4.11 (c)). The carbon-coated electrode achieved an  $R^2$  value of 0.9754, followed by the acid bleached and flame oxidised electrodes with  $R^2$  values of 0.9659 and 0.9384, respectively. These results indicate that all three modified electrodes were able to detect toxic pollutants with high linearity. The current decreased proportionally with increasing COD, showing that the system effectively captured the inhibitory effect of thinner on microbial activity. In comparison, the response to sodium acetate (Figure 4.11 (a)) showed moderate linearity. The carbon-coated electrode achieved an  $R^2$  value of 0.8773. The flame oxidised and acid bleached electrodes had lower values of 0.717 and 0.7351. Acetate is a simple organic compound that is rapidly metabolized by electrogenic microbes, which explains the strong response in current generation. However, microbial saturation or kinetic limitations at higher concentrations may have affected the linear relationship.

Ethanol testing (Figure 4.11 (b)) produced the weakest correlations overall. The carbon-coated and flame oxidised electrodes had  $R^2$  values of 0.5891 and 0.4102, while the acid bleached electrode recorded an extremely low value of 0.078. These low correlations suggest unstable sensor signals under ethanol exposure, which was due to microbial inhibition at high concentrations. Ethanol can impair electron transport pathways and lead to erratic current output, which reduces the predictive value of COD-current relationships. Additionally, nutrient limitation within the tank can also restrict the growth of electroactive biofilm, leading to partial biofilm detachment or heterogeneous metabolic activity across the electrode surface (Y. Wang et al. 2022), which weakens linear correlations. Furthermore, COD represents total oxidisable organic matter and does not distinguish between biodegradable and non-biodegradable fractions (Pluciennik-Koropczuk et al. 2020; Wu et al. 2014). Hence, under inhibitory

or stressful conditions, a substantial portion of the measured COD may remain biologically inaccessible, which causes COD variation and microbial electron transfer.

In the case of POME (Figure 4.11 (d)), the carbon-coated electrode again showed a strong linear response, with an  $R^2$  of 0.9598. The flame oxidised and acid bleached electrodes followed with  $R^2$  values of 0.8388 and 0.6464. POME contains complex organic compounds that require longer microbial adaptation and breakdown. Despite this, the carbon-coated sensor maintained good signal stability and tracking of COD levels. It is important to note that this test was conducted after nine months of continuous MES operation, yet the correlation remained strong. This outcome highlights the long-term durability and performance consistency of the carbon-coated electrode.

The overall trend shows that the carbon-coated SS304L electrode consistently produced high current output and maintained good linearity with COD in both toxic and organic-rich environments. This demonstrates its potential as a reliable and accurate biosensing material. Flame oxidised electrodes also performed well in some cases but showed more variability. Acid bleached electrodes appeared to perform better in toxicity tests but failed to deliver consistent results across other pollutant types.

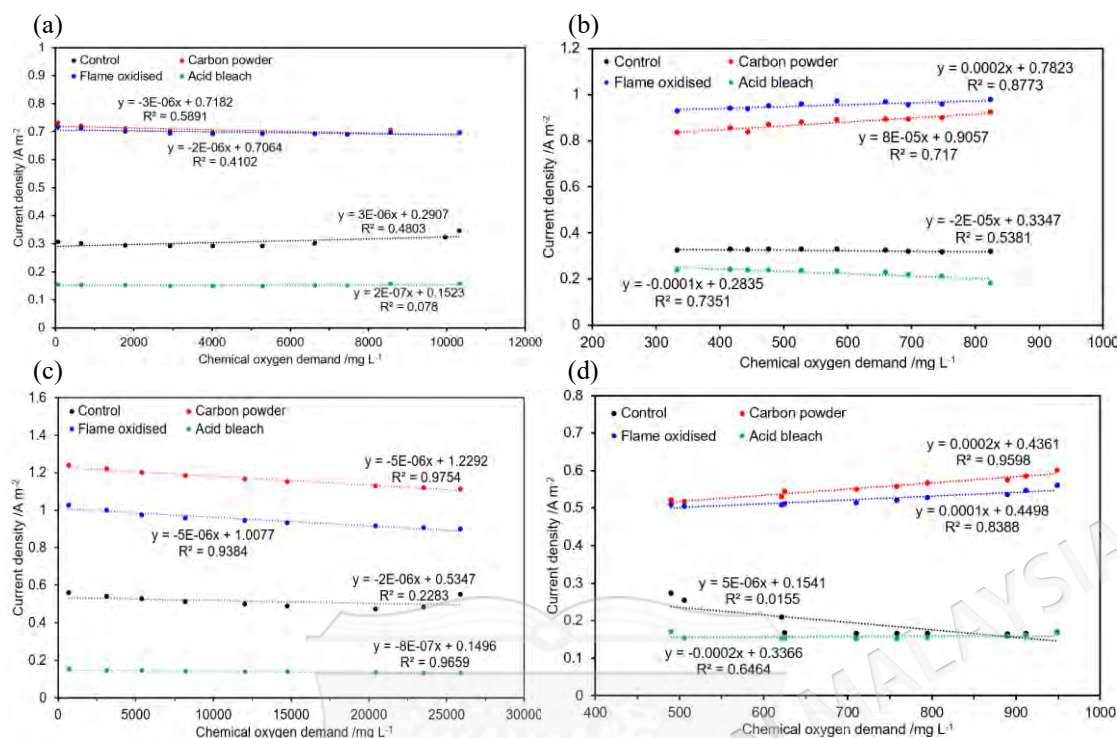


Figure 4.11 Correlation data between current density and COD values for (a) sodium acetate, (b) ethanol, (c) thinner, and (d) POME.

The variation in correlation strength,  $R^2$  between current density and COD across different pollutant tests is primarily governed by substrate biodegradability, microbial compatibility, and biofilm stability at the electrode surface. High correlations like those observed for sodium acetate and POME on the carbon-coated and flame-oxidised electrodes indicated that effective coupling between organic matter availability and microbial electron transfer, where changes in COD are proportionally reflected in the electrochemical response. Sodium acetate is readily biodegradable and directly metabolised by electroactive microorganisms, which resulted in a stable and linear COD–current relationship. In contrast, low correlations observed during ethanol and thinner tests reflect microbial inhibition and signal instability causing current output to fluctuate independently of COD variation. These effects were further amplified on electrodes with unfavorable surface properties, where weaker biofilm attachment and higher interfacial resistance reduced the consistency of electrochemical signals. Overall, strong COD–current correlations were achieved when biodegradable substrates were paired with electrode surfaces that supported stable biofilm formation and efficient

electron transfer, whereas low correlations arose from substrate toxicity, limited bioavailability, and disrupted bioelectrochemical activity.

The results confirm that the carbon-coated electrode provides both high responsiveness and excellent correlation with COD across a range of pollutants. It was particularly effective in detecting both biodegradable and toxic compounds. The strong linearity, even after long-term operation, supports its suitability for real-time wastewater monitoring applications. In contrast, flame oxidised and acid bleached electrodes showed moderate or inconsistent performance and may require further optimization.

#### 4.2.5 Summary

In summary, surface-modified SS304L electrodes (carbon-coated, flame oxidised, and acid bleached) were employed as anodes for MESe and evaluated using a custom-built water circulation system to enhance biocompatibility during biofilm development and improve sensing performance for wastewater monitoring. Among the tested materials, the carbon-coated anode consistently demonstrated superior performance across all pollutant tests, indicating its robustness and versatility under diverse wastewater conditions. For readily biodegradable substrates such as sodium acetate, the carbon-coated MESe exhibited rapid step-wise current responses that closely followed COD variations, which reflected efficient microbial oxidation and electron transfer. During the ethanol test, although high concentrations imposed inhibitory effects on microbial activity, the carbon-coated anode maintained a more stable electrochemical response than the control and acid bleached electrodes, suggesting greater resilience of the biofilm–electrode interface. In the POME test, the carbon-coated MESe showed a delayed yet substantial increase in current density due to the complexity of the pollutant's composition, which demonstrated its ability to support microbial acclimation and sustained electron generation under high and persistent COD conditions. In contrast, exposure to thinner resulted in current suppression across all sensors. However, the carbon-coated MESe still exhibited clearer signal differentiation compared to poorly performing electrodes, highlighting its sensitivity to both biodegradable and inhibitory organic loads.

Quantitatively, the carbon-coated MESe achieved peak current densities of up to  $1.3 \text{ A m}^{-2}$ , corresponding to approximately 2.6 times higher output than the unmodified control MESe, and exhibited the strongest correlation between current density and COD across all pollutant types with  $R^2$  values reaching up to 0.9754 (Figure 4.11 (c)). These performance advantages are strongly supported by SEM and AFM analyses, which revealed that the carbon coating produced a highly rough, porous, and heterogeneous surface morphology with pronounced peak–valley features. Such surface roughness is particularly favourable for biofilm formation, as it increases available surface area, provides physical anchoring sites for microbial cells, and promotes retention of extracellular polymeric substances, thereby facilitating stable biofilm establishment. Unlike the flame-oxidised electrode, where a compact oxide layer limited conductivity despite improved adhesion, or the acid bleached electrode, where excessive etching produced irregular but poorly structured roughness that hindered biofilm stability, the carbon-coated surface achieved an optimal balance between surface roughness, electrical conductivity, and biocompatibility. Collectively, these findings demonstrate that the carbon-coated anode provides the most suitable surface environment for electroactive biofilm development and efficient electron transfer, making it the most effective and reliable anode material for MESe applications across a wide range of wastewater pollutant scenarios.

#### **4.3 MICROBIAL FUEL CELL SENSOR (MFCSE)**

Building on the superior performance of the carbon-coated MESe, the next phase of sensor development focused on a multi-array tubular-type microbial fuel cell sensor (MFCSe), incorporating a novel air-cathode design for this application. Unlike the previous system where surface modifications were applied to the anode, this configuration retained carbon-based electrodes, specifically carbon cloth while emphasizing cathode-side enhancements to improve the oxygen reduction reaction and operational efficiency. Air-cathodes were used in MFCSe, which eliminates the need for an external power supply due to the cathodes being exposed to atmospheric oxygen. The MFCSe design not only leverages the biofilm compatibility and conductivity advantages of carbon materials but also addresses challenges such as cathodic limitations, fouling, and maintenance. The following section outlines the

physicochemical analysis, electrochemical characteristics, startup data, and pollutant response performance of the air-cathode MFCSe under various wastewater conditions.

#### 4.3.1 Physicochemical analysis

##### a. FESEM-EDX analysis

Figure 4.12 shows the FESEM images and EDX spectrum of the electroactive microbial biofilm developed on the unmodified carbon cloth anode. The formation of biofilm is a fundamental requirement for the MFCSe, similar to that of MESe. In Figure 4.12 (a), at a low magnification of 50x, the overall structure of the carbon can be observed and the interwoven bundles of carbon fibers create a highly porous matrix that facilitates the attachment of electroactive microbes. This porosity is a key physical characteristic of the electrode as it provides a large surface area for microbial colonization and allows efficient flow of wastewater and nutrients through the electrode, which are essential for sustained microbial activity. On the other hand, at a higher magnification of 1,000x, the FESEM image confirms the presence of a developing microbial community. The surface of the individual carbon fibers is no longer bare but was instead covered with a thin, layered material (Figure 4.12 (b)). Embedded within this film and attached to the fibers are numerous rod-shaped microorganisms, a common morphology for electroactive bacteria (Wu et al. 2018; Yadav et al. 2020). This composite layer of microorganisms and their surrounding extracellular EPS constitutes the electroactive biofilm, forming the bioanode.

The presence of this biofilm is crucial as these microorganisms are responsible for the oxidation of organic matter in the wastewater, transferring electrons directly to the carbon anode to generate an electrical signal that serves as the sensor's output. The visible cracks in the biofilm layer are typical phenomenon resulting from the dehydration process during sample preparation for FESEM imaging. The EDX spectrum in Figure 4.12 (c) further confirms the successful biofilm formation by analyzing the elemental composition of the anode surface. The high percentage of carbon (81.9%) is expected from the carbon cloth substrate and the microbial biomass. The significant presence of oxygen (15.4%) is attributed to the organic components of

the biofilm, particularly the polysaccharides and proteins that make up the EPS (Hasan et al. 2024; Karygianni et al. 2020). Furthermore, the detection of sodium (Na), chlorine (Cl), and a small amount of sulfur (S) provides important chemical evidence that the biofilm's composition reflects the surrounding wastewater environment (Subhadra. 2022). The porous and high surface area of the carbon cloth anode provided an excellent substrate for microbial colonization without any prior surface modification, thus validating its suitability as an anode material for your microbial electrochemical application (Al-Badani et al. 2024; Gandu et al. 2020).

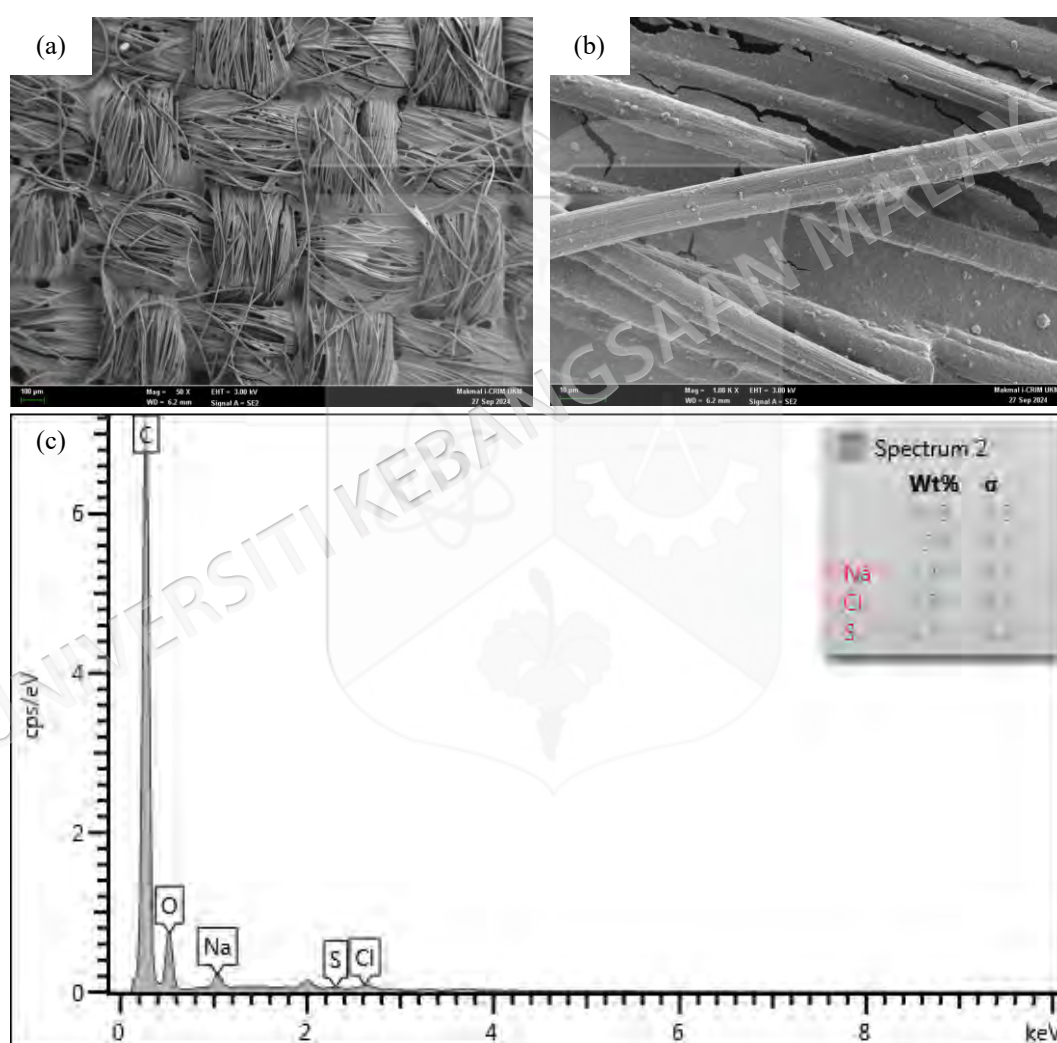


Figure 4.12 FESEM images of biofilm formation on plain carbon cloth anode at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.

The FESEM images in Figure 4.13 provide a detailed look into the physical structure of the air-cathode before the pollutant tests were conducted. At a magnification

of 50x, the carbon cloth was uniformly covered with a continuous, cracked coating (Figure 4.13 (a)). This coating is the active layer made of polytetrafluoroethylene (PTFE), carbon black powder (CB), and platinum on carbon (PtC). The cracks were common results of the drying process as the hydrophobic PTFE binder contracts (Ito et al. 2015).

The presence of micro-scale cracks within the PTFE–carbon black–Pt/C active layer can influence gas and liquid transport in ways that are relevant to cathode performance. Cracks in gas diffusion layers and catalyst layers have been shown to provide additional pathways for reactant access and product removal, effectively reducing diffusion resistance and facilitating oxygen transport to catalytic sites (Li et al. 2024). This allows efficient oxygen reduction in fuel cell and air-breathing cathode systems. Furthermore, this layered design is a widely used method in bioelectrochemical systems to create a gas diffusion layer, which prevents the liquid anolyte from leaking through the cathode while still allowing atmospheric oxygen to permeate the surface (Horst et al. 2016; Salvian et al. 2024).

Based on Figure 4.13 (b), the individual carbon cloth fibers were completely covered in a porous layer made of fine, particulate material. This layer consists of conductive carbon black particles bound by the PTFE matrix. Platinum nanoparticles are dispersed throughout this layer but are too small to be seen at this magnification, which was verified. This complex surface is specifically engineered to maximize the triple phase boundary where the gas (oxygen), liquid (electrolyte), and solid (catalyst) phases meet (Pan et al. 2024). As research has shown, a well-defined TPB is critical for enabling the efficient ORR (Mohapatra et al. 2024; Zhong et al. 2022), which is a major bottleneck in MFC technology. This structural analysis confirms that the cathode's surface is fundamentally different from the anode's, which is designed for microbial growth. While the anode's success depends on the formation of a living biofilm, the cathode's performance relies on this carefully engineered abiotic and hydrophobic layer. These images provide strong evidence that the cathode was successfully fabricated with a structure designed to facilitate the oxygen reduction reaction, which is a crucial step towards validating the overall performance of the MFCSe system.

The EDX spectrum in Figure 4.13 (c) confirms the elemental composition of the air-cathode and provides insight into the distribution of its constituent materials. The high weight percentages of carbon (C) and fluorine (F) are consistent with the expected composition, where carbon originates from the carbon cloth and carbon black support, while fluorine is associated with the PTFE binder (Senna et al. 2012). The presence of platinum (Pt) at approximately 11.6 wt% corresponds to the Pt/C catalyst layer; however, given that platinum is dispersed as nanoparticles on a carbon support, its signal intensity is inherently limited by particle size, surface coverage, and the interaction volume of the EDX analysis. Oxygen (O) was detected at a relatively low level, which may arise from surface-adsorbed species or inorganic deposits formed during operation (Z. Li et al. 2015). Overall, the EDX results are consistent with the designed cathode composition, while also highlighting the surface-sensitive nature and compositional limitations of EDX in resolving nanoscale catalyst distributions.

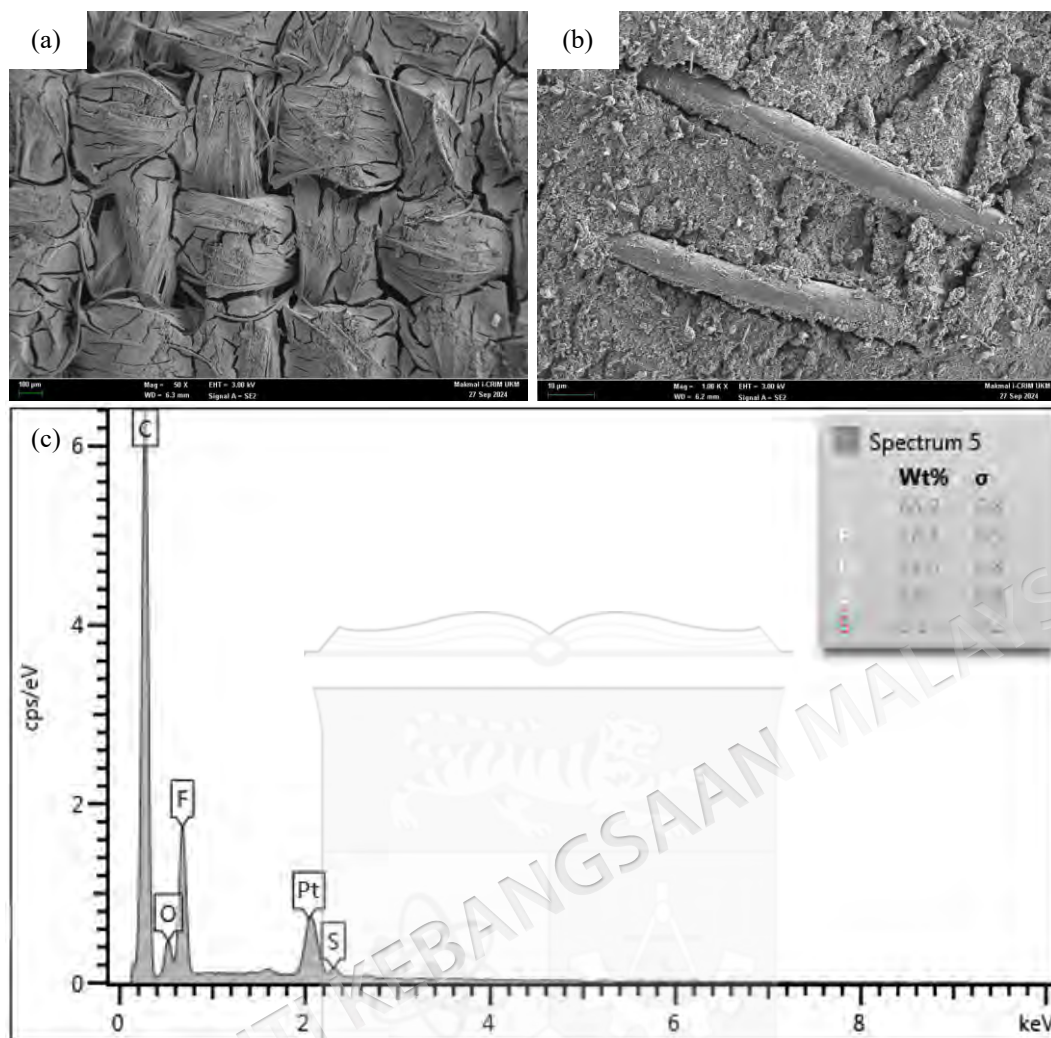


Figure 4.13

FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at 0.5 mg cm<sup>-2</sup> loading rate of PtC on carbon cloth air-cathode (before pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.

Figure 4.14 shows the FESEM images and elemental analysis for the air-cathode coated with PTFE/CB/PtC at 2.0 mg cm<sup>-2</sup> loading rate of PtC. The FESEM images provide clear visual evidence that the intended higher catalyst loading rate was achieved. The air-cathode fabricated with a 2.0 mg cm<sup>-2</sup> loading rate of PtC (Figure 4.14 (b)) possessed a significantly thicker, denser, and more heavily cracked coating when compared to the 0.5 mg cm<sup>-2</sup> sample (Figure 4.13 (b)). The denser layer significantly buries the individual carbon fibers, suggesting that the increased material has created a more robust gas diffusion layer. The EDX analysis further supports this comparison by revealing subtle yet important differences in elemental composition. Both spectra show carbon and fluorine as the dominant elements. However, the higher loading sample

exhibits a slightly higher carbon content and a reduced fluorine percentage. This shift in elemental ratio can be attributed to the increased proportion of carbon originating from the carbon black and platinum-on-carbon components in the catalyst layer, which has been consistently reported in studies examining carbon-supported catalyst systems using EDX analysis (Kobayashi et al. 2022; Wang et al. 2010). Another notable finding from the EDX spectra is the higher percentage of Pt in the  $2.0 \text{ mg cm}^{-2}$  sample (13.3 wt%), compared to the  $0.5 \text{ mc cm}^{-2}$  sample (11.6 wt%). Despite a fourfold increase in the nominal Pt/C loading rate, the measured platinum content increased by only approximately 2 wt%, indicating that the EDX signal does not scale linearly with catalyst loading.

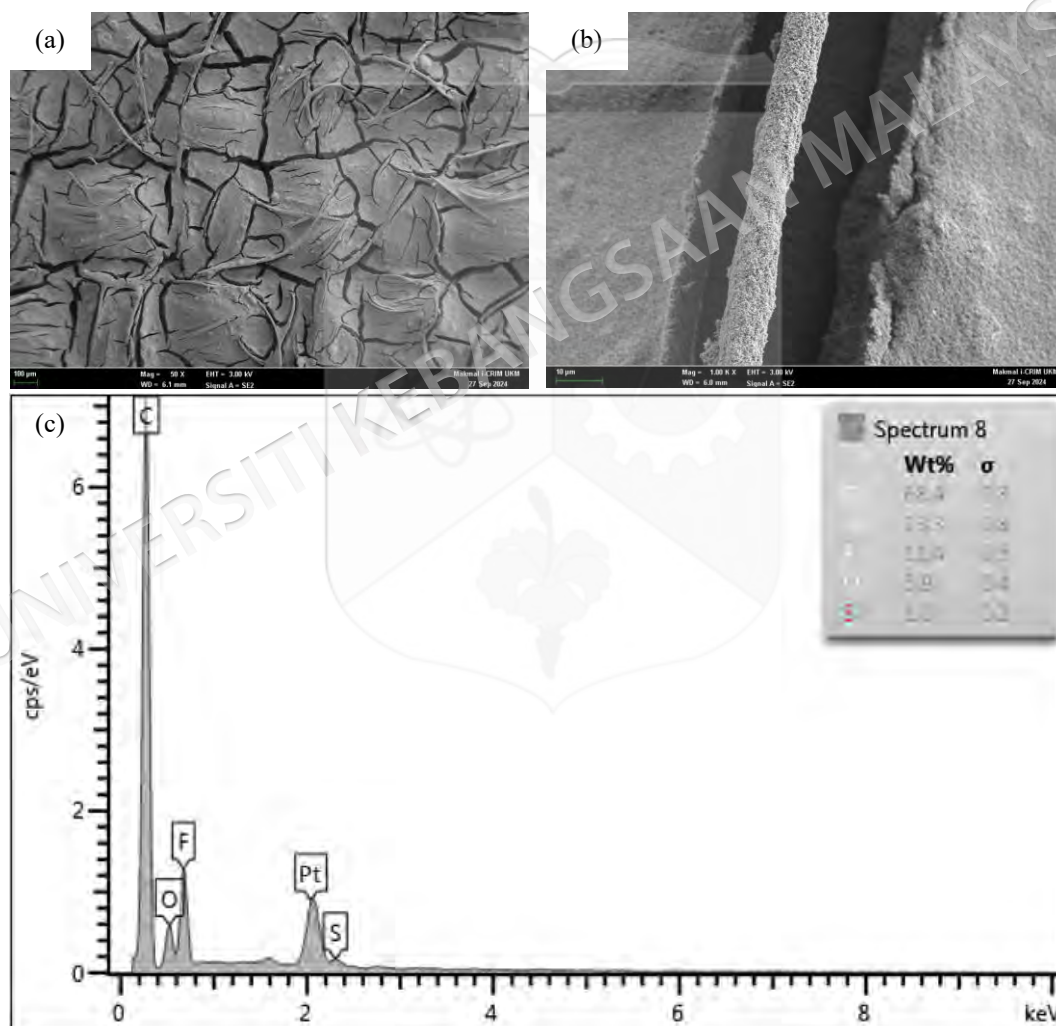


Figure 4.14 FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at  $2.0 \text{ mg cm}^{-2}$  loading rate of PtC on carbon cloth air-cathode (before pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.

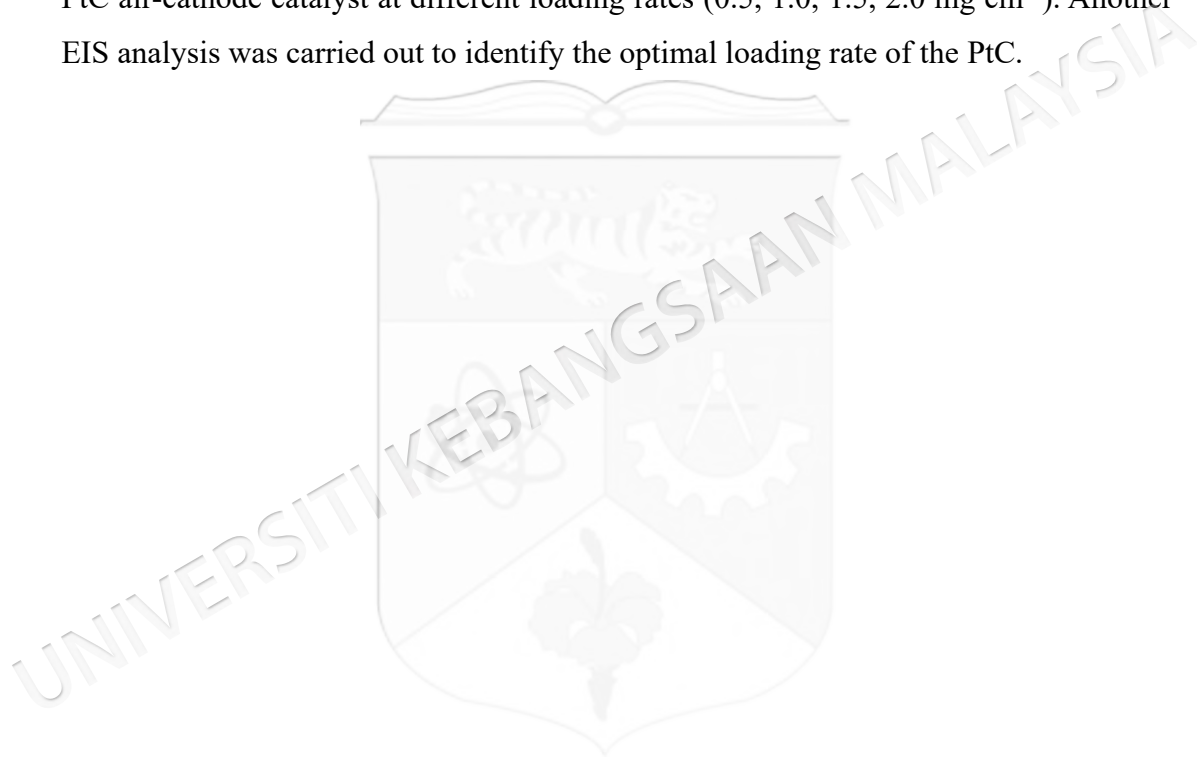
Based on Figure 4.15 (a), the smooth and cracked PTFE layer from the air-cathode before testing can no longer be observed. After the biosensing operations, the air-cathode coated surface became rough and covered in irregular crystalline structures, with long and distinct rod-like deposits. This visual transformation is a direct result of the cathode's continuous reduction. The crystalline deposits were salt precipitates that have accumulated on the cathode surface, which is a common phenomenon in bioelectrochemical systems known as cathodic scaling (Hasson et al. 2016; Yang et al. 2022). The formation of cathodic scales is a significant form of fouling and restricts the diffusion of oxygen to the reaction sites, which ultimately affects long-term performance of the biosensor. The EDX spectrum for the post-test cathode provides clear elemental evidence that aligns with the observed morphological changes (Figure 4.15 (c)). The significant increase in oxygen content from 4.9% to 42.9% at the air cathode may not solely reflect participation in ORR, but also the formation of calcium carbonate ( $\text{CaCO}_3$ ) precipitates on the cathode surface (Santini et al. 2017). In air-breathing cathode MFCs, alkalization near the cathodic sites during ORR can increase local pH, promoting carbonate precipitation in the presence of calcium ions, a phenomenon that has been reported to produce inorganic carbonate scale on cathode surfaces and influence electrochemical performance (Edwardsen et al. 2020; Santini et al. 2017). This accumulation is the primary reason for the substantial decrease in the original components C and F since their signals are now being masked by the new mineral deposits. In summary, the combined FESEM and EDX analysis provides strong evidence that the cathode was actively engaged in the ORR, but that its surface was also significantly altered by mineral scaling. This highlights a critical challenge to the long-term reliability of MFC-based sensors in real-world wastewater conditions.

Figure 4.15 FESEM images of carbon cloth air-cathode with PTFE/CB/PtC at 0.5 mg cm<sup>-2</sup> loading rate of PtC on carbon cloth air-cathode (post-pollutant testing) at (a) 50X magnification, (b) 1000X magnification, and its (c) EDX spectrum.

Similar to the previous sample (Figure 4.15), air-cathode with  $2.0 \text{ mg cm}^{-2}$  loading rate of PtC also exhibited a dramatic transformation of the cathode surface from a smooth surface to a rough and heavily scaled surface as shown in Figure 4.16 (a). At 1000X magnification (Figure 4.16 (b)), the air-cathode is dominated by dense, crystalline deposits, which appeared to be even more substantial than those on the lower loading sample (Figure 4.15 (b)). The EDX spectrum provides elemental evidence for these changes. The O content obtained is higher (47.8%) than that of  $0.5 \text{ mg cm}^{-2}$  PtC sample (42.9%), confirming the hypothesis that the higher PtC loading rate led to a more active oxygen reduction reaction and a greater accumulation of oxygen-containing species (Figure 4.16 (c)). This is a key indicator of improved performance. However,



PTFE/CB/PtC, and PTFE/CB/PANI). It can be obviously seen that the PTFE/CB/PtC possessed the lowest polarization resistance ( $R_p$ ) among other materials due to its high electrical conductivity. A significantly low  $R_p$  is important for the charge transfer process, especially in water quality monitoring as it eases electrical signal transfers and assures a more sensitive detection range. The solution resistance ( $R_s$ ) ranged from 45-60  $\Omega$  for all the biosensors, the similar  $R_s$  values indicated that they are indeed immersed in the same water source in a pipeline. Through the EIS analysis, the most optimum material among the air-cathodes is the PTFE/CB/(PtC) and this material was selected for further optimization in this project, which included replacing all the biosensors with PtC air-cathode catalyst at different loading rates (0.5, 1.0, 1.5, 2.0 mg cm<sup>-2</sup>). Another EIS analysis was carried out to identify the optimal loading rate of the PtC.



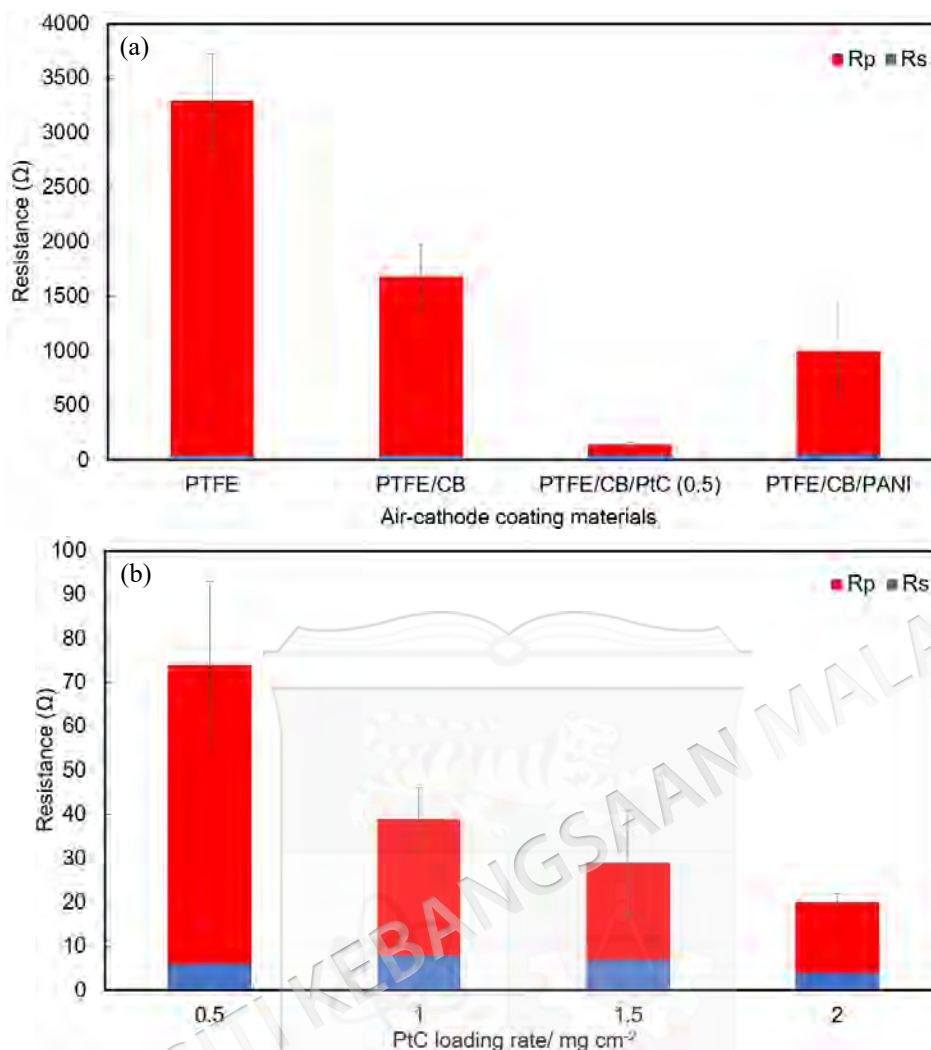


Figure 4.17 Average cell resistances of MFCSe with (a) different air-cathode materials and (b) PTFE/CB/PtC air-cathode with different PtC loading rates.

#### 4.3.3 Enrichment

The enrichment process and enrichment data are crucial for studying biofilm development. The biosensors were enriched using a 12-hour stock feeding regime until a stable and repeatable electrical signal was achieved, characterized by recurring wavelike current patterns corresponding to successive feeding cycles. This behaviour is consistent with the metabolic dynamics of electroactive bacteria, which are known to exhibit rapid growth and high metabolic activity within typical 12–16 hour cycles (Yamamoto et al. 2023; Zajdel et al. 2018), during which substrate availability stimulates electron transfer and current generation. The repeating signal profiles therefore indicate the establishment of an active and responsive electroactive biofilm.

In Figure 4.18, the signal exhibited continuous upward movement until day 18, at which point 10 L were withdrawn from the 20 L reservoirs and replaced with fresh tap water. The signal appeared to stabilize over the following two weeks, from day 20 to day 35, although the signal from PtC continued to trend upward, resembling previous patterns. This marks our initial trial-and-error attempt to identify the early response of the enriched biosensor. As the biofilm matured and electroactive microbial activity increased, a progressive rise in current density was observed, indicating a reduction in the internal resistance of the microbial fuel cell. Under these conditions, the initially selected higher-value resistor became less suitable for accurately capturing voltage changes, as the resulting voltage drop diminished and reduced measurement sensitivity. Consequently, a lower-value resistor was introduced to better match the evolving electrical characteristics of the system and maintain an appropriate measurement range (Koók et al. 2021; Pinto et al. 2011). The transition from a 1000  $\Omega$  to a 500  $\Omega$  resistor allowed higher current flow and improved signal resolution during long-term monitoring, particularly from day 36 to day 48 of operation. This adjustment reflects the dynamic nature of bioelectrochemical systems, where the optimal external resistance is not fixed but must be adapted to changes in biofilm development and internal resistance to ensure reliable and sensitive signal acquisition (López Zavala et al. 2023). It can be observed that the highest average current density was consistently achieved by the PTFE/CB/(PtC) biosensor, indicating that PtC is the suitable air-cathode catalyst among other materials. In contrast, the control sample with only a PTFE coating showed the lowest current density values recorded within the 48 days of enrichment period.

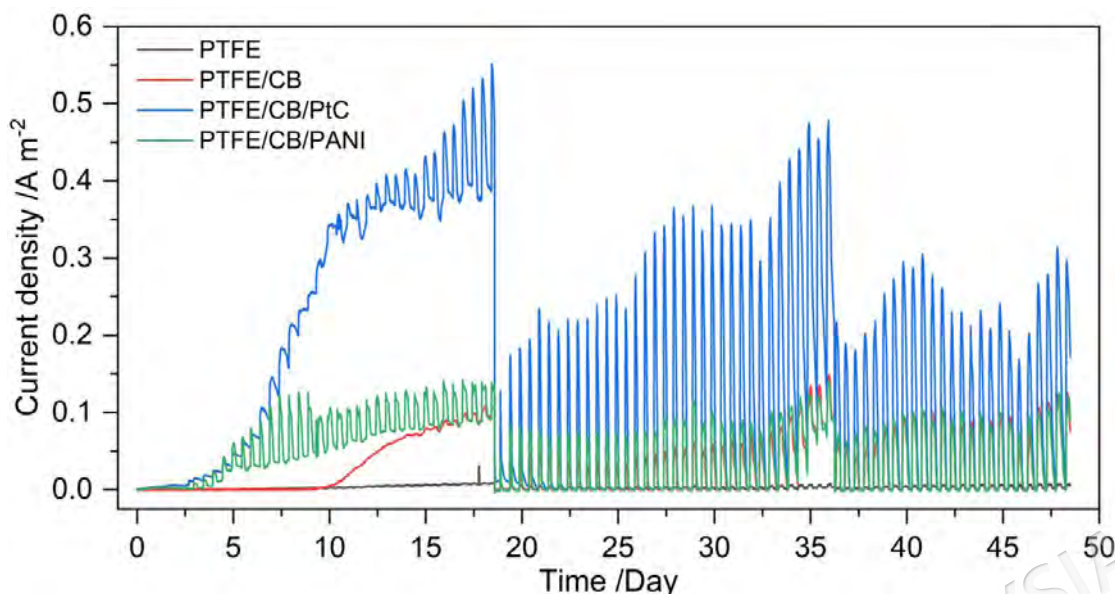


Figure 4.18 Enrichment data.

#### 4.3.4 Pollutants testing

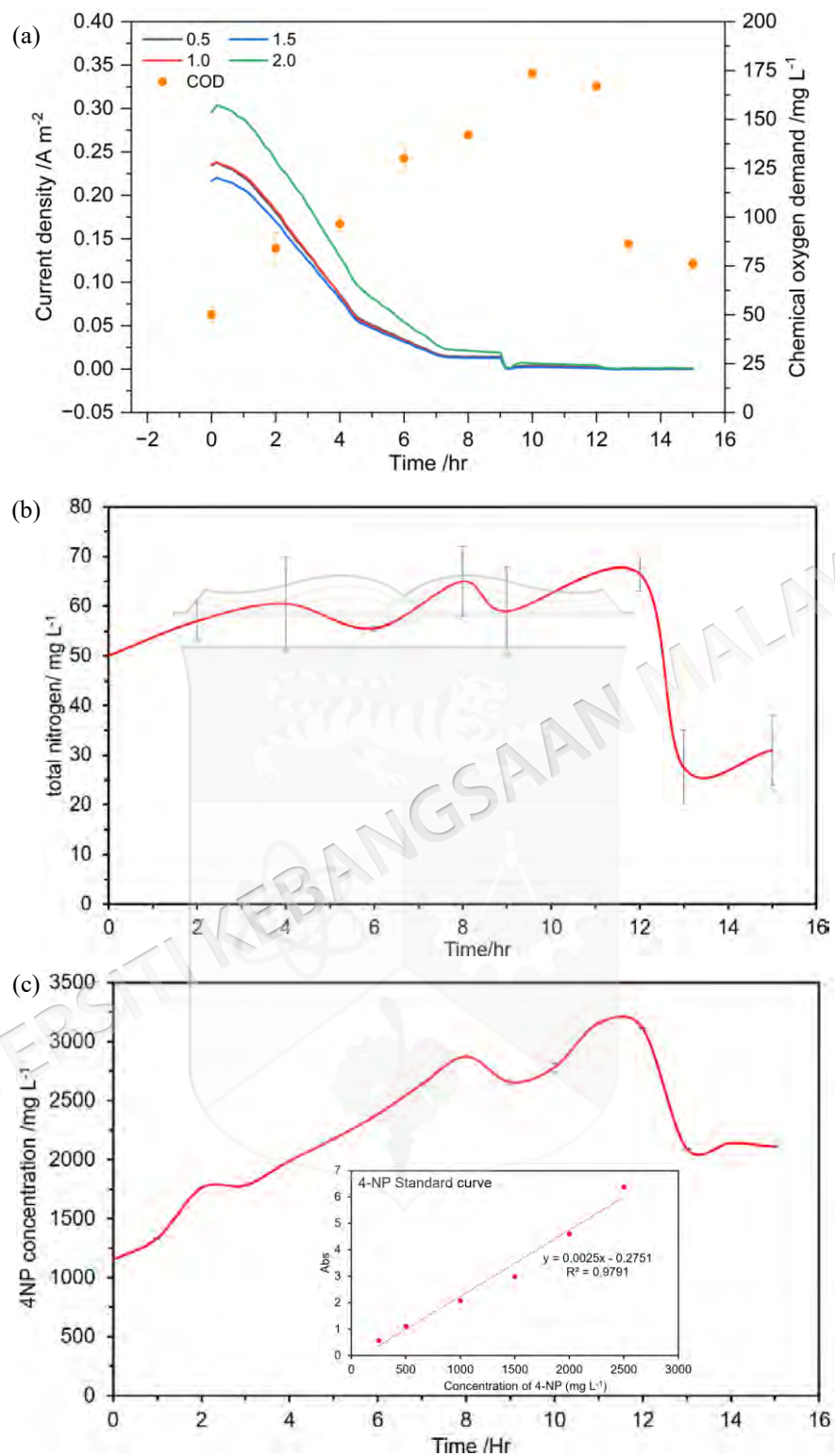
##### a. 4-Nitrophenol (4NP)

Once a stable signal response of up to 3 cycles was achieved for all biosensors, they are considered ready to be tested with the model pollutants. Generally, 4-nitrophenol (4-NP) is a toxic substance that is primarily produced from manufacturing and processing industries which may cause groundwater contamination, and hence, it was selected as a model pollutant to test for the MFC-biosensor's signal. The 4-NP pollutant testing was carried out for 15 hours (Figure 4.19).

During the spike test, the biosensor's current densities were seen to decrease gradually for the first 9 hours. Since 4-NP is toxic, the increment of 4-NP injection increased the toxicity levels of the influent, which disrupts the microorganism's activity in producing electrons. Therefore, the current density signal dropped when more toxic 4-NP was injected. At the 7th and 8th hour, a flat current density signal was observed, which indicated that the microorganisms had reached the maximum inhibition level. During the B2 - dumping test, a huge amount of 4-NP was injected in one shot into the reservoir, which caused the reservoir to be highly toxic. This further led to the sudden current density drop from the 9th hour to the 12th hour (Figure 4.19 (a)), suggesting

that most of the microorganisms attached to the biosensors were entirely inhibited. When microorganisms are exposed to high levels of toxic compounds, cell death is often accompanied by denaturation or degradation of extracellular and surface-associated proteins involved in adhesion. These binder proteins are key components of the EPS and they lose their functionality under toxic stress (Mahto et al. 2022), thereby reducing the ability of microbial cells to attach to electrode surfaces and destabilizing the biofilm structure (Hasan et al. 2025). After the dumping test was done, all the pumps were stopped to execute the next procedure which was the dilution test. The procedure involved draining half of the water from the setup reservoir and replacing it with fresh tap water instantly. For the 12th to 15th hours, almost zero current density was observed due to the loss of the microorganisms from the toxicity shock.

During the pollutant testing for 4-NP, liquid samples were also collected for further physicochemical analysis such as pH, conductivity, chemical oxygen demand (COD), total nitrogen (TN), and 4-NP concentration of liquid samples (Figure 4.19 (b-f)). Generally, COD concentration is an indicator of the amount of oxygen required to treat the incoming wastewater, it is the most common and direct method used to measure the concentration of pollutants present in a water body. By using 360 nm as the measurement wavelength, an absorbance vs. concentration standard curve for 4-NP was prepared as well (See the inserted graph in Figure 4.19 (c)). Lastly, total nitrogen (TN) test was carried out to obtain the sum of all the nitrogen forms, which is useful to serve as a verification data since 4-NP also consist of nitrogen groups (Figure 4.19 (b)). Based on Figure 4.19 (b-f), the trend on each graph showed an increase of concentration followed by a sudden drop of pollutants concentration due to the dilution test.



... to be continued

... continuation

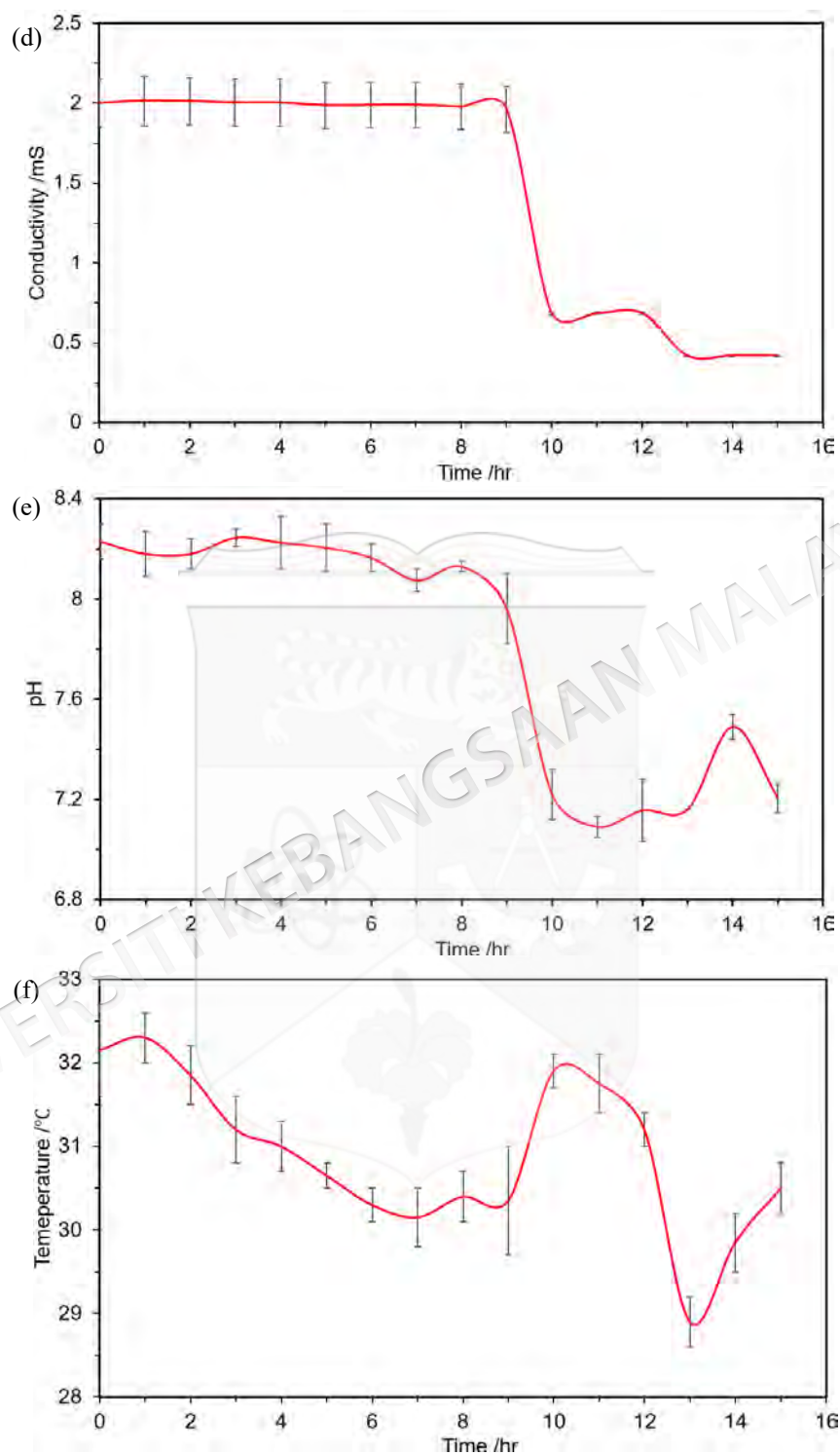


Figure 4.19

(a) Signal profile and COD during pollutants testing for 4-Nitrophenol (4NP), average chemical analysis data (b) Total nitrogen (TN), (c) Injection concentration, (d) conductivity, (e) pH, and (f) temperature.

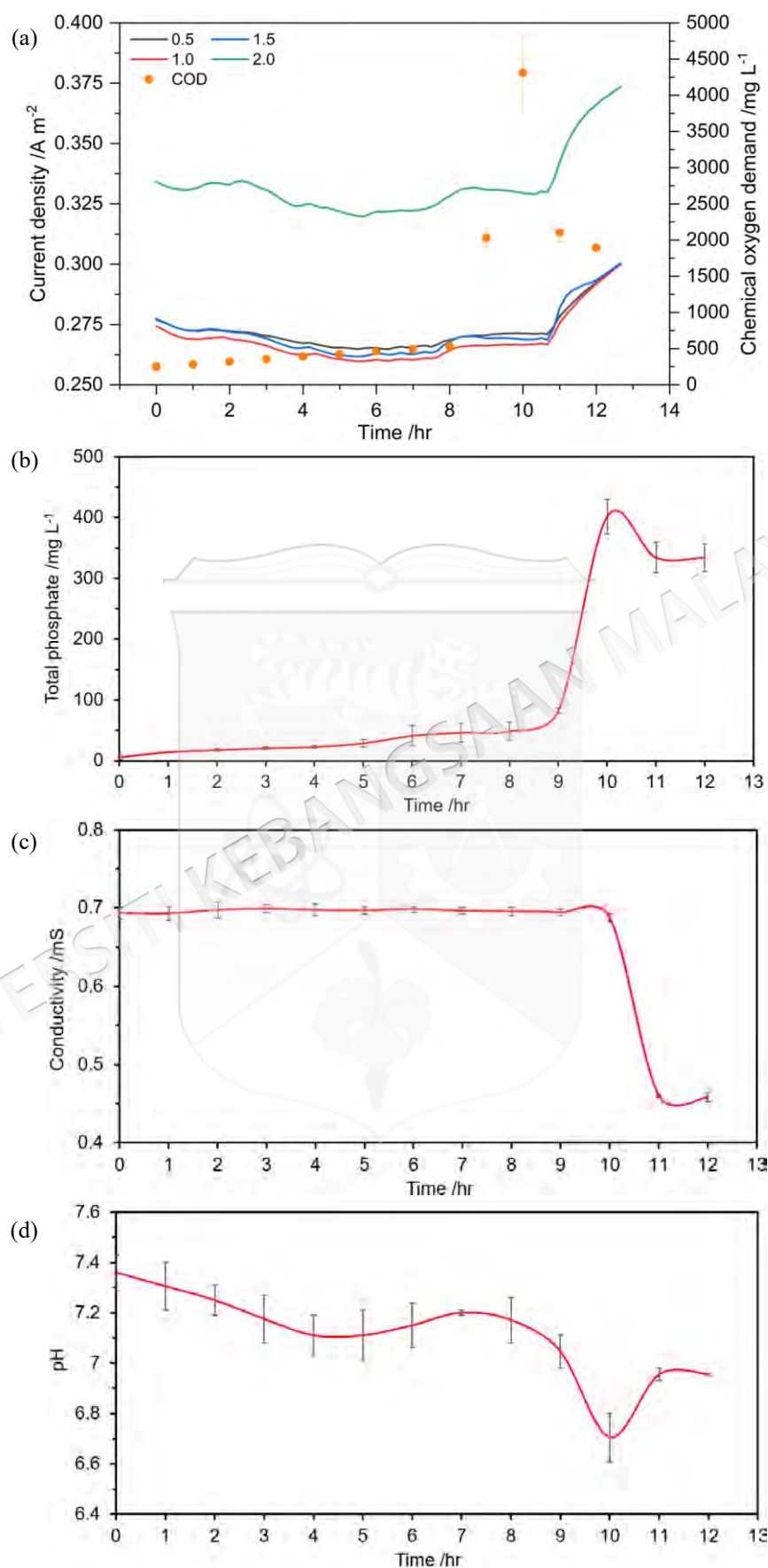
## b. Malathion

Figure 4.20a shows the overall current density profile during the testing of the pollutant. In this project, 2 mL of Malathion 570E insecticide was injected every hour for 8 hours during the incremental test, followed by 20 mL of the same insecticide during the dumping test. The concentration of the injected chemical was also tracked using water COD (Figure 4.20 (a)) and total phosphate reagent test (Figure 4.20 (b)). Despite the sudden increase of the COD and total phosphate in the system, all the biosensors did not show significant changes towards the chemical. Only a slight decrease of the current density signal profile was observed during the incremental test, suggesting that malathion is indeed harmful towards the biofilm on the anode. There was no significant decrement of the signal as malathion is a complex molecule up to 10 carbons with a chemical formula of  $C_{10}H_{19}O_6PS_2$ .

Therefore, microbes often require longer time to degrade complex molecules compared to simpler ones. This is because complex molecules have a more intricate structure and may require multiple enzymatic reactions to break down. Additionally, the degradation of complex molecules may involve the cooperation of multiple microbial species, leading to the formation of a microbial community (Cao et al. 2022). However, the microbes were still exposed directly to malathion's toxic properties, which led to the decrease of the current densities. Similarly, malathion's direct contact with human's skin potentially causes systemic poisoning if a significant amount is absorbed through the pores (Koca et al. 2024). After 11 hours, half of the 20L reservoir was replaced with fresh tap water, in which the amount of malathion in the system was also halved. Hence, an increase of the current density signal was observed during the dilution phase due to the lesser inhibition by the remaining malathion (Figure 4.20 (a)). Similar to previous results, the air-cathode with  $0.5 \text{ mg cm}^{-2}$  PtC loading rate produced the highest current density compared to the rest.

In addition to the electrochemical response, variations in bulk water properties were also observed during the malathion test, as shown in Figure 4.20 (c–e). The conductivity profile (Figure 4.20 (c)) remained relatively stable during the incremental injection phase, indicating that the hourly addition of malathion did not substantially

alter the ionic strength of the system. However, a sharp decline in conductivity was observed following the dilution step, which is consistent with the replacement of half of the reservoir volume with fresh tap water and the consequent reduction in dissolved ions. The pH profile (Figure 4.20 (d)) exhibited a gradual decrease during the dumping phase, followed by partial recovery after dilution, suggesting that malathion introduction and its associated formulation components contributed to localized acidification, which may further stress electroactive microorganisms and suppress metabolic activity (Rauglas et al. 2016). Temperature changes (Figure 4.20 (e)) were minimal during most of the test but dropped noticeably after dilution, reflecting the introduction of cooler tap water into the system. This sudden temperature reduction may have imposed additional physiological stress on the biofilm (H. Li et al. 2015; Tian et al. 2024), as electroactive microbial activity is highly temperature-dependent. In short, these fluctuations in conductivity, pH, and temperature likely compounded the toxic effects of malathion and contributed to the muted and delayed current density response observed during this test.



... to be continued

... continuation

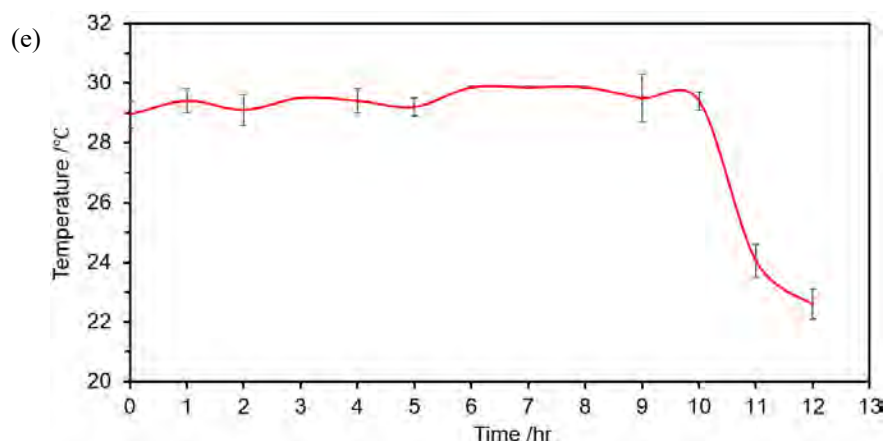
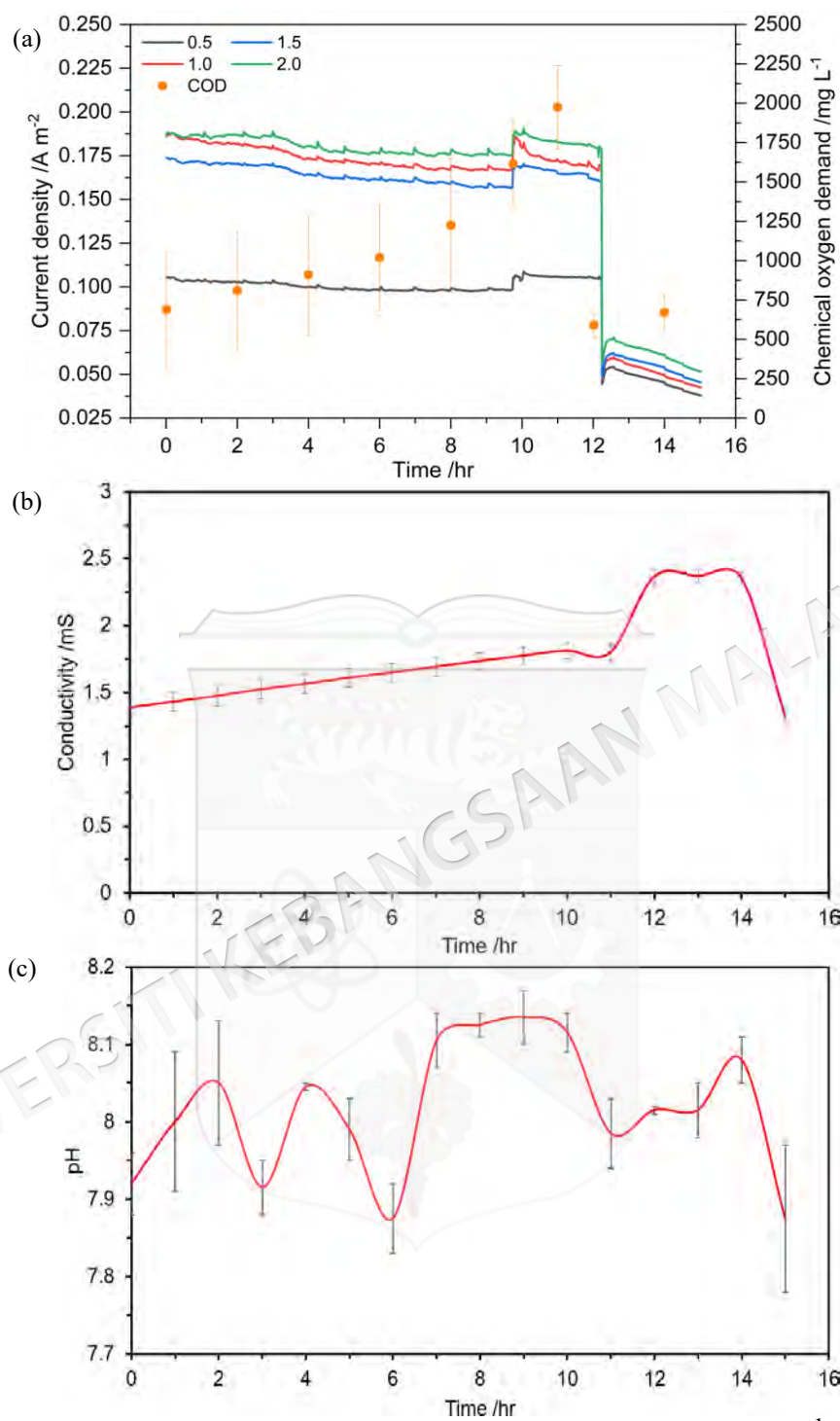


Figure 4.20 (a) Signal profile and COD during pollutants testing for malathion, average chemical analysis data (b) Total phosphate, (c) conductivity, (d) pH, and (e) temperature.

### c. Palm oil mill effluent (POME)

Figure 4.21 shows the biosensor data for the POME pollutant testing. The test was carried out after the signal has achieved stability from the previous 4-NP test. The POME pollutant test was carried out for 15 hours, which included the spike test, dumping test, and dilution test. As compared to the previous 4-NP pollutants, the signals from Figure 4.21 (a) showed tiny spikes for the first 9 hours which was associated with the POME injection at every hour. Here, a similar trend was witnessed where the  $0.5 \text{ mg cm}^{-2}$  sample was still generating the highest current density values throughout the test. Next, an obvious large spike was detected at the 10<sup>th</sup> hour, which was due to the dumping of a huge amount of POME into the reservoir. The signal was maintained from the 10<sup>th</sup> to the 12<sup>th</sup> hour before dropping drastically when the dilution test was carried out. When comparing POME with the 4-NP test, the initial signal responses did not promote any significant inhibition which indicated that POME does not possess any levels of toxicity that could be poison to the microorganisms. Based on Figure 4.21 (a) and Figure 4.21 (b), the average COD values and the conductivity of the liquid samples collected showed similar trends. The COD and conductivity values increased slowly during the first 9 hours, followed by a sharp increase during the dumping test, and lastly a steep drop of voltage due to the dilution test. Overall, the COD and conductivity of the liquid samples collected showed a close relationship with the biosensor cell voltages.

The pH and temperature profiles recorded during the POME test further support the observed electrochemical behaviour (Figure 4.21 (c-d)). Throughout most of the test, the pH remained within a relatively narrow alkaline range ( $\approx 7.9$ – $8.1$ ), indicating a buffered system that is favourable for electroactive microbial activity. Minor pH fluctuations were observed during the incremental and dumping phases, but no abrupt acidification occurred, suggesting that the organic constituents of POME did not impose significant chemical stress on the biofilm. Similarly, the temperature profile showed gradual variations associated with environmental conditions and liquid handling, with a brief increase during the dumping phase followed by a decrease after dilution. These temperature changes did not coincide with any immediate suppression of current density, indicating that microbial metabolism remained largely resilient. In contrast to the POME test, both 4NP (Figure 4.19) and malathion (Figure 4.20) tests exhibited stronger disturbances in electrochemical output despite comparable or lower COD levels. This difference can be attributed to the toxic nature of malathion and 4-NP (Godain et al. 2020), which directly inhibit microbial enzymes and disrupt electron transfer pathways causing signal suppression (Guler et al. 2016). Although POME is characterized by high organic loading consists predominantly of biodegradable organic compounds that require longer microbial adaptation (Bala et al. 2015), it does not exert acute toxicity. As a result, the MFCSe exhibited sustained current generation and stable signal behaviour during POME exposure, highlighting its suitability for monitoring complex organic wastewater as opposed to toxic industrial pollutants.



... to be continued

... continuation

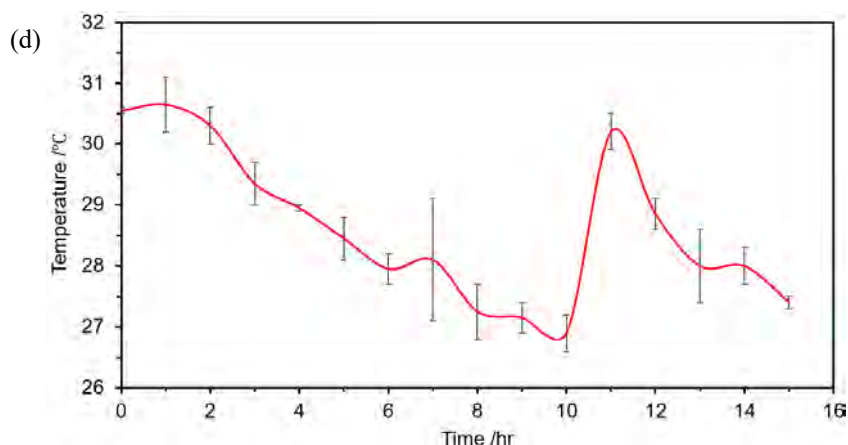


Figure 4.21 (a) Signal profile and COD during pollutants testing for POME, average chemical analysis data (b) conductivity, (c) pH, and (d) temperature.

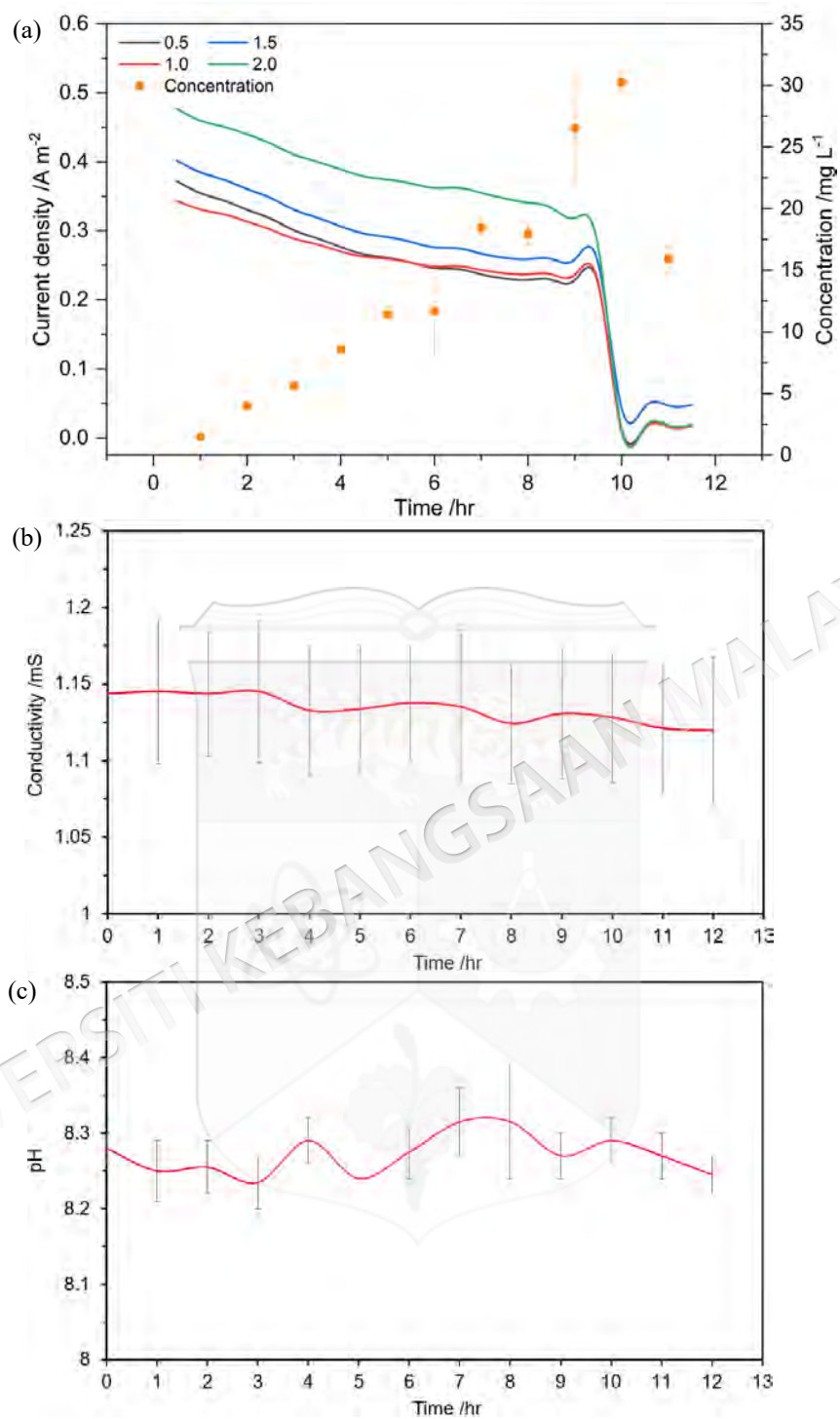
#### d. Methyl orange (MO)

Figure 4.22 (a) presents the average current density response of the MFCSe during methyl orange (MO) pollutant testing, together with the corresponding injection concentration profile. During the incremental injection phase, a gradual and continuous decrease in current density was observed across all electrodes. This behaviour indicates progressive inhibition of electroactive microbial activity as the MO concentration increased. Unlike readily biodegradable substrates, MO is an azo dye with a stable aromatic structure that is resistant to rapid microbial degradation (Kishor et al. 2021). As a result, the dye does not contribute effectively to anodic electron generation and instead interferes with microbial respiration and extracellular electron transfer processes in the MFCSe (Khan et al. 2021; Xie et al. 2024). During the dumping test, the current density stabilised and no further significant change was detected despite additional MO input. This plateau indicates that the reservoir medium had become saturated with MO, such that further increases in dye concentration no longer produced additional inhibitory effects on the anodic biofilm. The absence of sharp current responses compared to biodegradable substrates such as sodium acetate (Figure 4.19 (a)) and POME (Figure 4.21 (a)) also hinted the inhibitory and non-bioavailable nature of MO toward MFC-based sensing.

The supporting physicochemical parameters shown in Figure 4.22 (b–d) further explain the muted electrochemical response observed during MO testing. Conductivity

remained relatively constant throughout the experiment, indicating that MO injection did not significantly alter the ionic strength of the electrolyte and that the observed current density changes were not driven by variations in solution resistance. Similarly, the pH profile exhibited only minor fluctuations within a narrow alkaline range, suggesting that MO addition did not impose substantial pH stress on the electroactive microbial community. In contrast, the temperature profile showed a gradual decline over time, which may have contributed to the overall reduction in current density by slowing microbial metabolic activity and electron transfer rates. However, the absence of abrupt temperature shifts indicates that thermal effects were secondary to the intrinsic toxicity and recalcitrant structure of MO. Overall, these results demonstrate that the MFCSe response to MO is governed primarily by dye-induced microbial inhibition and limited biodegradability, rather than by changes in bulk solution chemistry.





... to be continued

... continuation

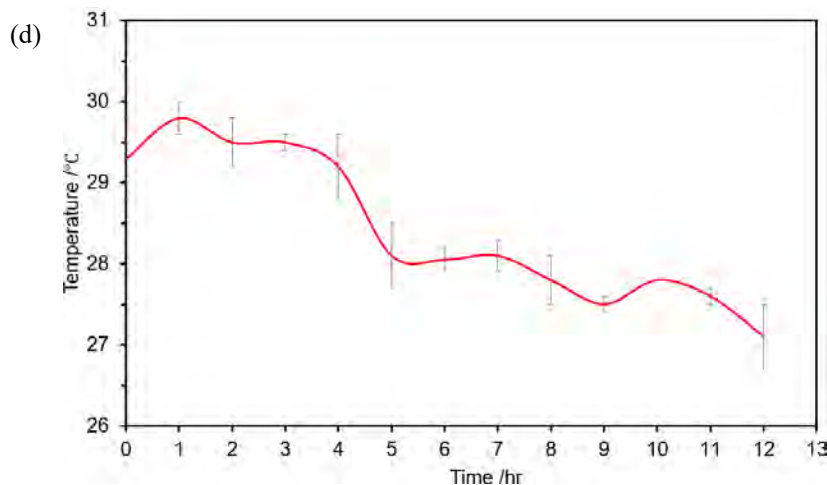


Figure 4.22 (a) Signal profile and injection concentration during pollutant testing for methyl orange, average chemical analysis data (b) conductivity, (c) pH, and (d) temperature.

#### 4.3.5 Correlation data

##### a. 4-Nitrophenol (4NP)

The purpose of performing the traditional chemical analysis for the water samples is to obtain results and compare them with the MFCSe signals. Correlation graphs can be plotted using both the biosensor voltage data and the results from chemical analysis. Figure 4.23 shows the correlation graphs plotted using each biosensor's data with those of chemical analysis. Based on Figure 4.23, the  $R^2$  values for 0.5, 1.0, 1.5, and 2.0 mg  $\text{cm}^{-2}$  samples when comparing current densities and COD values were 0.8471, 0.8264, 0.8248, and 0.8290, respectively. This result indicated that the cell voltages generated were closely associated with the presence of the 4-NP presence in the water body. MFCSe with 2.0 mg  $\text{cm}^{-2}$  PtC air-cathode possessed the highest correlation values, while the others performed very similarly to one another within the range of 0.824 to 0.829. Also, 2.0 mg  $\text{cm}^{-2}$  samples recorded the steepest gradient in both graphs, suggesting that the rate of change of average cell voltage to the injection of pollutants was the highest among the rest. This also means that the biosensor with 2.0 mg  $\text{cm}^{-2}$  PtC loading rate possessed the highest sensitivity.

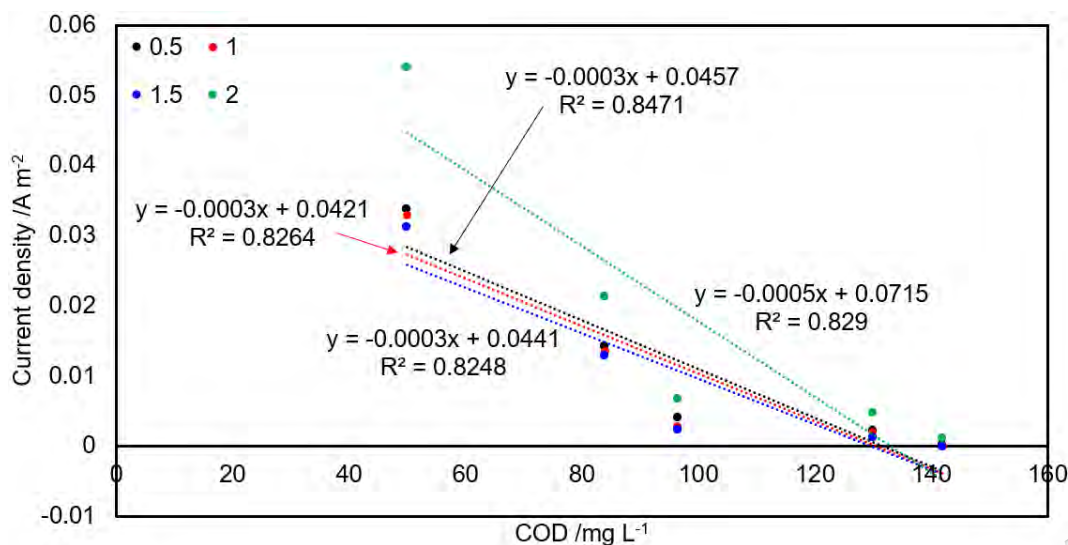


Figure 4.23 Linear correlation graphs depicting the averaged current density in responses to COD during 4NP pollutant test.

#### b. Malathion

Figure 4.24 shows the correlation data between the average current densities of the biosensors with COD and total phosphate results. The current densities of the biosensors correlated with COD recorded R<sup>2</sup> values of 0.8906 (0.5 mg cm<sup>-2</sup> PtC), 0.9105 (1.0 mg cm<sup>-2</sup> PtC), 0.9655 (1.5 mg cm<sup>-2</sup> PtC), and 0.9492 (2.0 mg cm<sup>-2</sup> PtC). The air-cathode with a 1.5 mg cm<sup>-2</sup> PtC loading rate demonstrated the best performance, which was evidenced by its R<sup>2</sup> value of 0.9655. While the other loading rates also showed strong correlations (R<sup>2</sup> values greater than 0.89), the 1.5 mg cm<sup>-2</sup> loading is statistically superior, as its output signal most consistently tracks changes in the organic load. The slight decrease in correlation observed at the highest loading rate of 2.0 mg cm<sup>-2</sup> (R<sup>2</sup> = 0.9492) suggests that a higher catalyst loading does not always result in a better sensor performance. This could be due to factors such as mass transfer limitations, where the densely packed catalyst layer inhibits the diffusion of substrates and oxygen, or the Pt catalyst particles becoming less accessible for the reaction. Therefore, the 1.5 mg cm<sup>-2</sup> loading represents the optimal balance for achieving both high catalytic activity and a strong, predictable linear response in detecting malathion.

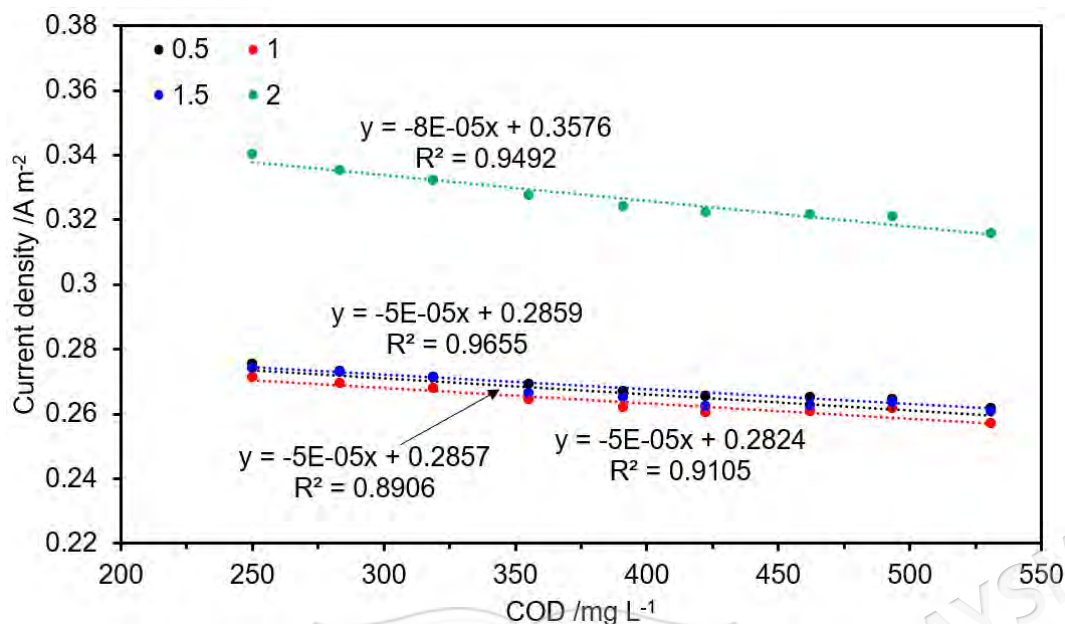


Figure 4.24 Linear correlation graphs depicting the averaged current density in responses to COD during malathion pollutant test.

### c. Palm oil mill effluent (POME)

Based on the provided correlation data for POME pollutants (Figure 4.25), the air-cathode with a  $1.5 \text{ mg cm}^{-2}$  PtC loading rate demonstrated the best performance for monitoring Chemical Oxygen Demand (COD). This is supported by the highest  $R^2$  value of 0.9364, which indicates the strongest and most reliable linear relationship between the biosensor's current density and the concentration of COD in the POME samples. While other loading rates also showed strong correlations, the decrease in the  $R^2$  value at  $2.0 \text{ mg cm}^{-2}$  ( $R^2 = 0.7701$ ) suggests a diminishing return. This may be due to the catalyst layer becoming too thick, which can hinder the diffusion of substrates and oxygen to the triple-phase boundary, thereby limiting the reaction kinetics. Therefore, the  $1.5 \text{ mg cm}^{-2}$  PtC loading represents the optimal concentration for achieving the best balance between catalytic activity and efficient mass transport for the accurate detection of COD in POME.

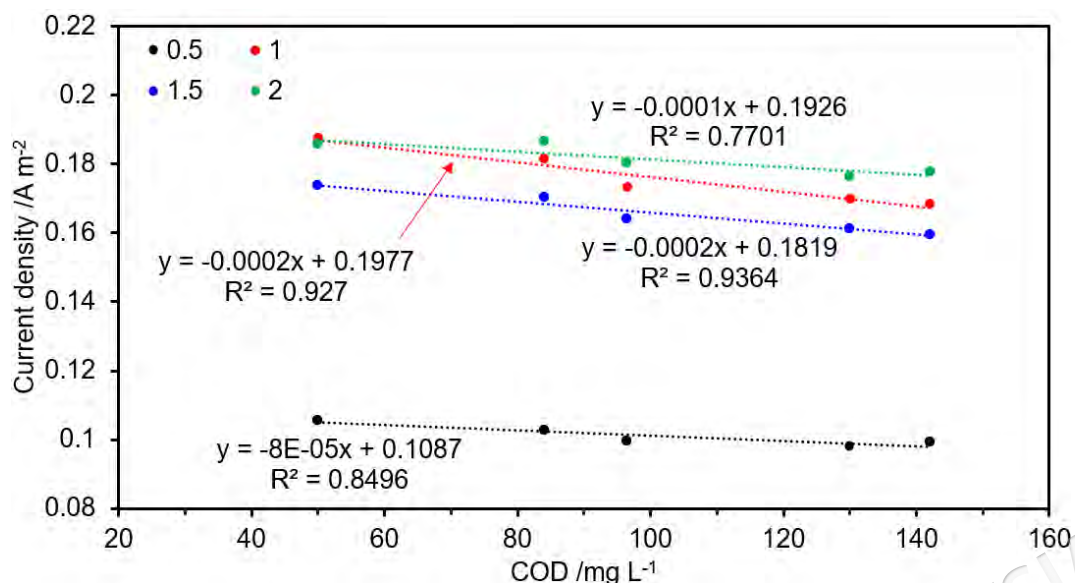


Figure 4.25 Linear correlation graphs depicting the averaged current density in responses to COD during POME pollutant test.

#### d. Methyl orange (MO)

As shown in Figure 4.26 (a), the data points for current density and POME pollutants form a nearly perfect straight line. The exceptionally high R<sup>2</sup> of 0.9478 by 2.0 mg cm<sup>-2</sup> confirms an extremely strong linear relationship. This is an ideal scenario for sensor calibration, as it allows for a simple and reliable conversion of the sensor's electrical signal into a corresponding pollutant concentration using a linear equation. Such a strong linear fit indicates that the biosensor's response is consistent and predictable across the tested concentration range.

Non-linear correlation describes a relationship where the rate of change is not constant and requires a curved line to accurately model the data. Figure 4.26 (b) demonstrates this relationship, where the data points follow a distinct curve rather than a straight line. By fitting a non-linear polynomial curve to the data, the R<sup>2</sup> values for each MFCSe increases above 0.9950 (0.9953 for 2.0 mg cm<sup>-2</sup>, 0.9959 for 1.5 mg cm<sup>-2</sup>, 0.9968 for 0.5 mg cm<sup>-2</sup>, and 0.9957 for 1.0 mg cm<sup>-2</sup>), indicating a slightly better fit than the linear model. This superior fit suggests that while a linear model is highly effective, the sensor's behavior is more accurately described by a non-linear function, likely due to saturation effects or mass transfer limitations at higher pollutant concentrations. Although more complex for calibration, a non-linear model provides a more precise and

robust method for predicting pollutant concentrations, especially when operating over a wide range.

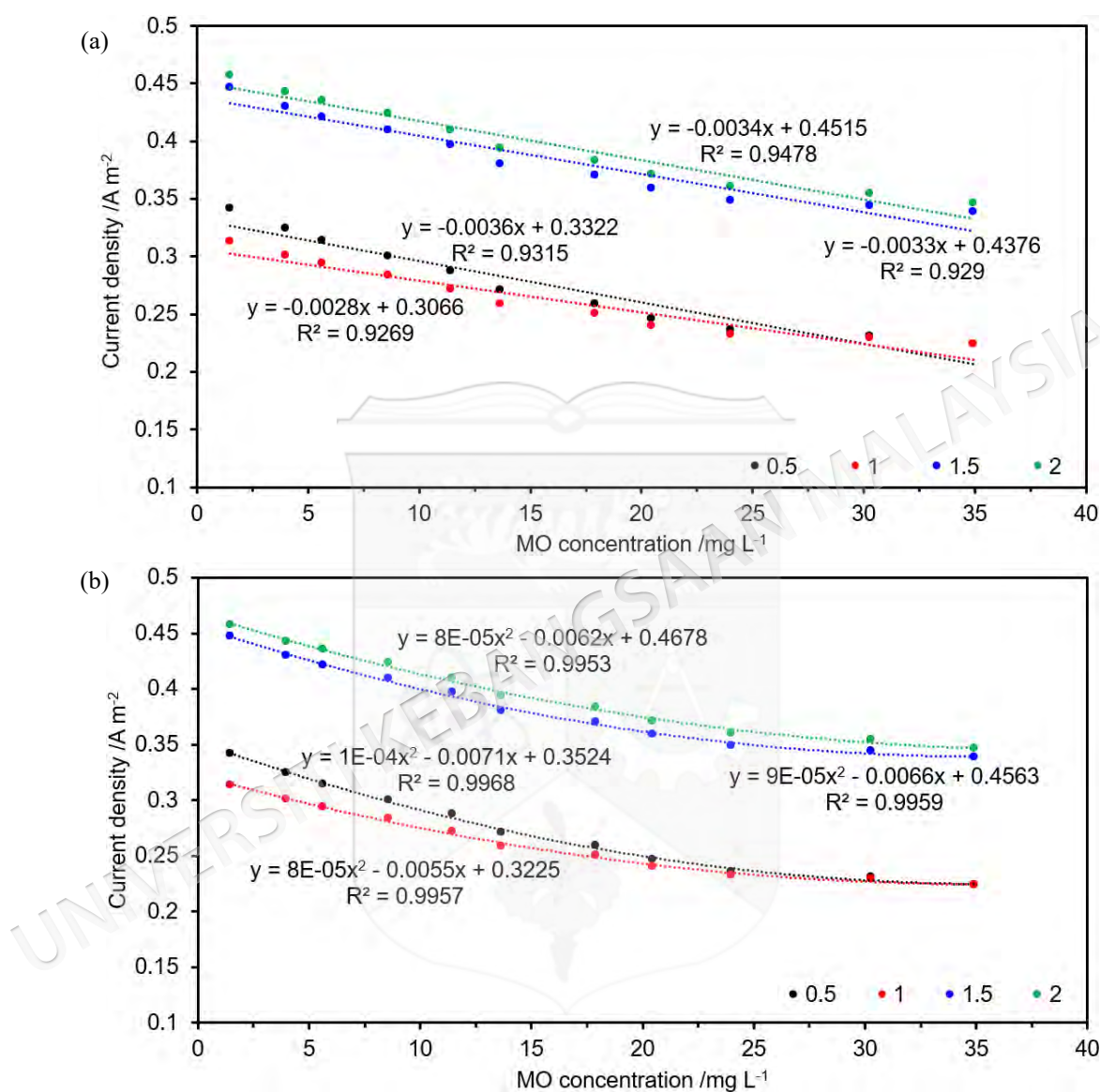


Figure 4.26 (a) Linear and (b) Non-linear correlation graphs depicting the averaged current density in responses to MO concentration during MO pollutant test.

#### 4.3.6 Microbial community analysis

Figure 4.27 shows that the major phylum found from the microbial community analysis are Proteobacteria (34%), Bacteroidota (26%), and Acidobacteriota (21%). Proteobacteria is a dominant class of bacteria in MFCs, are crucial for the functioning

and efficiency of these bioelectrochemical systems. In the context of MFC-based biosensors, various strains within the Proteobacteria phylum, including *Geobacter*, *Xanthobacter*, and *Pseudomonas*, are instrumental in power generation, pollutant removal, and electron transfer processes. Their versatility and prevalence in MFCs make them significant in driving the bioelectricity generation and bioremediation capabilities of MFC-based biosensors (Cui et al. 2019). Bacteroidota plays a crucial role in microbial fuel cell-based biosensors. While there is limited specific research on Bacteroidota in MFC-based biosensors, their ability to contribute to bioremediation processes in MFCs suggests their potential role in detecting toxic compounds or biochemical oxygen demand (BOD) in biosensor applications (Zhou et al. 2017). However, further studies are needed to establish their specific contributions and interactions within MFC-based biosensors. Acidobacteriota are prominently found in the microbial community of MFCs, as they are commonly found in soils near the bank of a lake (Bretschger et al. 2010). While their specific role in MFC-based biosensors is not well-studied, their adaptation and potential contribution to the electroactive biofilm formation within these systems highlight their potential for participating in biosensor processes. One study had showcased their role in efficient bioelectricity generation in a urine-fed MFC system (Obata et al. 2020).

Since these three major phyla (Proteobacteria, Bacteroidota, and Acidobacteriota) were found in the same electrode during 16S rRNA genetic sequencing, it suggests a diverse and complex microbial community in the microbial fuel cell (MFC). These phyla are known to interact and form synergistic relationships in MFCs, enhancing the overall performance of the system. For instance, Proteobacteria, such as *Geobacter* and *Rhodocyclaceae*, have been observed to cooperate and promote the bioelectricity generation in MFCs (Jiaxin Li et al. 2022). This cooperation is sustainable and enhances the positive connections within electroactive consortia, promoting internal electron transfer (IET) inside the system. In the phylum Bacteroidota, *sphingobacteriales* play a role in the oxidation of organic matter and the production of electricity. The bacteria in this order are known to be capable of extracellular electron transfer, which is an important mechanism for the functioning of MFCs (Ramanaiah et al. 2021), more importantly in biosensor application. Acidobacteriota's presence in the microbial community of MFCs signifies its adaptation and potential contribution to the



oxygen, the MFCSe also demonstrated consistent current generation and superior sensitivity under various wastewater conditions. Its scalable and durable design further supports its suitability for real-world deployment. Building on this performance, the next phase focuses on integrating Internet of Things (IoT) technology to enable real-time data transmission and automated alarm notifications for rapid water quality monitoring and response.

#### **4.4 Alarm notification system with Internet of Things (IoT)**

In the following section, the incorporation of real-time data transmission and an automated alarm notification framework is presented, demonstrating how the MFCSe platform can be transformed into a smart, self-alerting biosensing system capable of rapid response to water quality anomalies in real-world settings.

##### **4.4.1 Performance of MFCSe integrated with mathematical models**

Change point detection method is based on the rate of change (ROC) of the current density [ $(\text{A m}^{-2})/\text{min}$ ] as the model compares each data point with the next data point. Hence, configuring the suitable ROC in this application is very important to prevent unnecessary alarm triggers. For example, when the alarm is set to trigger when the  $\text{ROC} > 0.0003$ , the alarm will only be triggered when a feeding occurs (Figure 4.28 (a)). However, if the alarm value is set to be too low such as  $\text{ROC} > 0.00003$  (one-tenth difference), even the surrounding noise caused the alarm to be triggered (Figure 4.28 (b)).

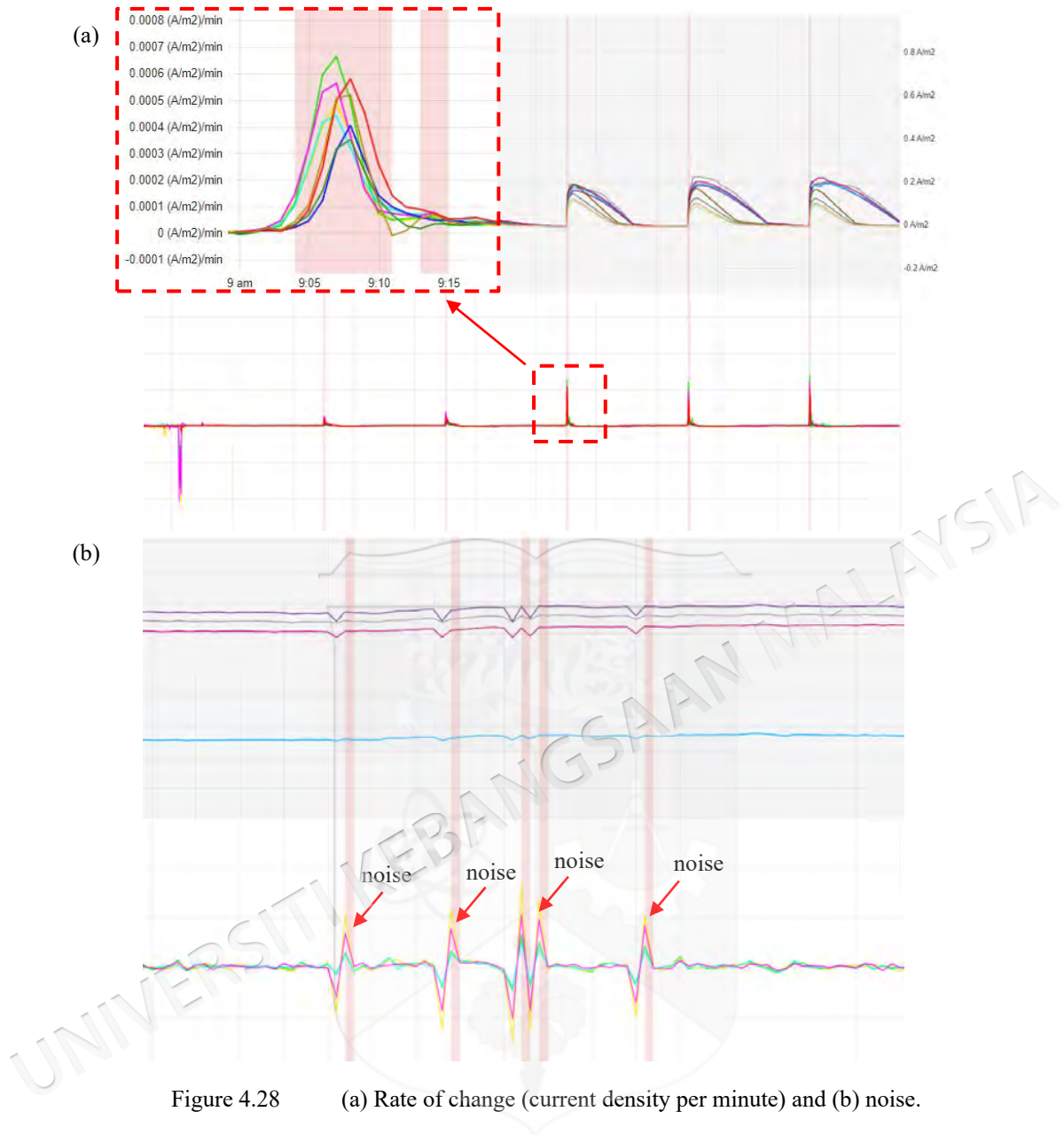


Figure 4.28 (a) Rate of change (current density per minute) and (b) noise.

Both anomaly (max and min thresholds) and change point detection (spike) mathematical models were verified when testing with POME. During the incremental test, small spikes were produced in the current density signal profile, which is suited for the change time detection (Figure 4.29 (a)). This is because the current density level did not shift abruptly. On the other hand, a pollutant's dumping test will cause the current density to shift greatly as seen in Figure 4.29 (b). Therefore, anomaly detection involving maximum and minimum thresholds are more suitable in this scenario. Based on these results, both detection methods should be used simultaneously to provide a more detailed and accurate feedback which allows better decision making by the users.

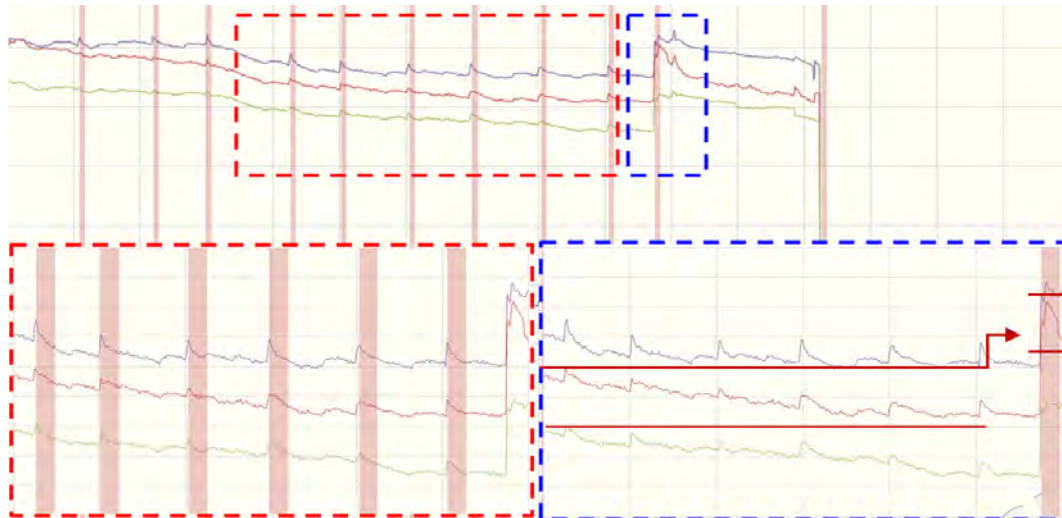


Figure 4.29 (a) Change point detection and (b) anomaly detection.

Lastly, the alarm triggered can be linked with IFTTT and WebHooks service to send a notification email to more than one user as shown in Figure 4.30.

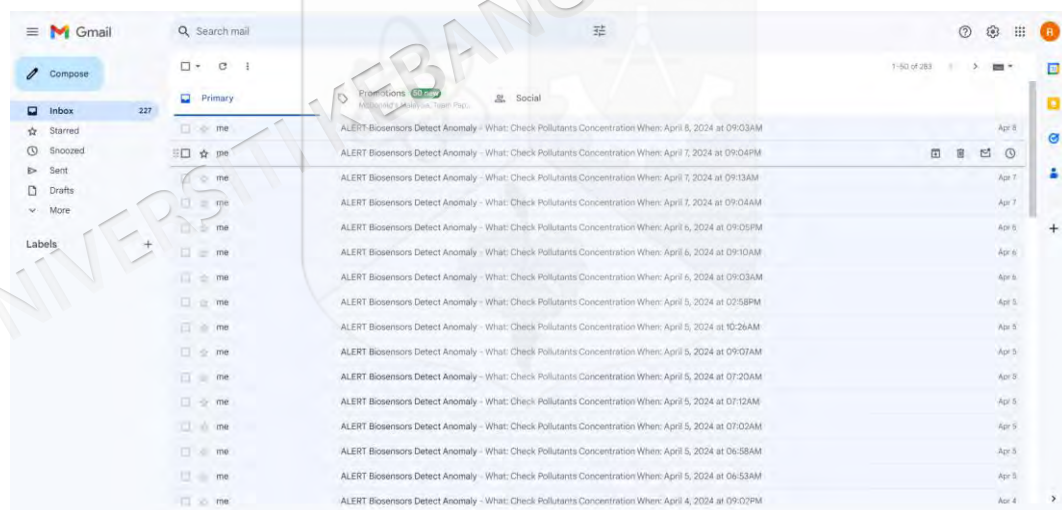


Figure 4.30 Notification email sent to the user when an alert is triggered.

#### 4.4.2 Summary

This section outlined the integration of real-time data transmission and automated alarm functionality into the MFCSe platform, enabling its operation as a smart and self-alerting biosensor. Two mathematical models were implemented: change point detection based on rate of change, and anomaly detection using defined threshold

values, both designed to capture signal variations indicative of pollutant events. The system was further enhanced through IFTTT and Webhooks, allowing instant email notifications to multiple users for timely response. These features significantly increase the practicality of the MFCSe in real-world applications, where continuous, autonomous monitoring is essential. The integration of these intelligent functions positions the MFCSe as a highly suitable tool for deployment in municipal wastewater treatment settings, where immediate detection of pollution events and system anomalies can improve operational decision-making and safeguard water quality. The following section discusses the on-site installation and performance of the fully integrated MFCSe system at a local wastewater treatment plant.

#### **4.5 ACTUAL MUNICIPAL WASTEWATER QUALITY DETECTION**

To evaluate its field readiness, the fully integrated MFCSe system, equipped with an air cathode, real-time IoT connectivity, and mathematical detection models, was deployed at a municipal wastewater treatment facility. This installation aimed to assess the sensor's ability to function reliably under actual operating conditions, continuously monitor influent quality, and trigger timely alerts during pollutant fluctuations or abnormal events. The following section presents the performance data collected during on-site operation.

##### **4.5.1 Detection of actual municipal wastewater**

Figure 4.31 (a) shows the biosensor's current profile during the microbial enrichment phase, Figure 4.31 (b) illustrates its operation mode after achieving a stable current output. During this phase, electrochemically active microbes were encouraged to colonize the anode surface, initiating the biofilm formation necessary for sensor activation. As shown in Figure 4.31 (a), microbial activity during enrichment followed a typical sigmoid growth pattern. After initial lag, the exponential phase displayed a sharp increase in current density, indicating active microbial attachment and colonization on the anode. This behavior aligns with previous findings (Yeo et al. 2024), which emphasized the role of functional electroactive microbes in enhancing biosensor responsiveness.

After approximately three months of continuous operation, Figure 4.31 (b) shows that MFCSe with  $1.5 \text{ mg cm}^{-2}$  PtC and MFCSe with  $2.0 \text{ mg cm}^{-2}$  PtC consistently generated higher and more stable current outputs compared to those with  $0.5$  and  $1.0 \text{ mg cm}^{-2}$  PtC loading rates. Notably, a stable daily or weekly current pattern is considered the baseline for normal biosensor operation. Deviations from this baseline, such as a significant drop in current may signal the presence of toxic contaminants or inhibitory compounds in the influent. These compounds can suppress microbial activity or cause microbial death, leading to reduced electron transfer and lower current output.



Figure 4.31

Data Collection during (a) microbial enrichment process and (b) monitoring operation after microbial growth.

The commissioning of the system monitoring was successfully carried out and is in line with the initial design specifications. The biosensor system has demonstrated reliable performance over time. During the continuous monitoring period from 4 to 15 March 2024, no irregularities were detected. Moreover, the biosensor data was consistent with the daily COD measurements conducted by IWRC between 10:00 AM and 11:00 AM, further validating the sensor's sensitivity and stability (Figure 4.32).

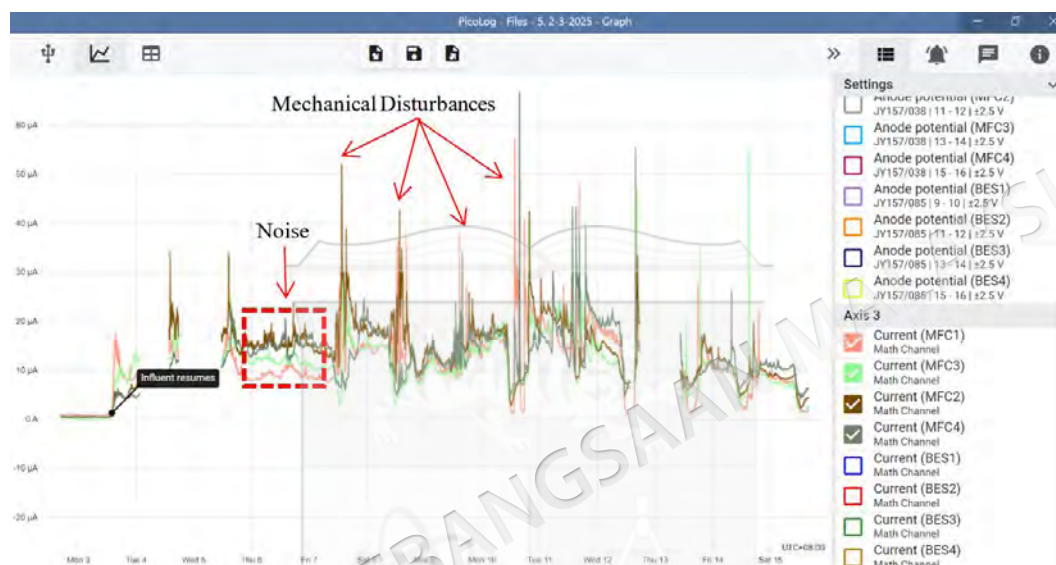


Figure 4.32 Mechanical disturbances and noise.

#### 4.5.2 Sensitivity

In general sensor science, sensitivity is defined as the ratio of output signal change to input analyte concentration change. Therefore, in this MFC-biosensor, the current output is directly related to the bioelectrochemical activity of electrogenic microbes, which in turn is influenced by substrate availability such as influence COD and BOD. The consistent amplitude and frequency of the electrical response suggest that the sensor can detect even minor perturbations in wastewater quality, with low baseline noise and good repeatability. The biosensor demonstrated notable sensitivity to changes in wastewater composition, a critical requirement for real-time environmental monitoring.

Initially, the biosensor exhibited a biofilm development phase, followed by repeatable wave-like current signals as fresh influent was introduced daily to the

treatment plant (Figure 4.33). These current variations, ranging from approximately 2  $\mu\text{A}$  to 70  $\mu\text{A}$ , reflect the sensor's responsiveness to fluctuations in the organic and ionic content of the wastewater stream. During the biofilm development phase, the current generated fluctuated from 2  $\mu\text{A}$  up to 70  $\mu\text{A}$ , and the signal slowly stabilized below 10  $\mu\text{A}$  after the biofilm had developed (Figure 4.33 (a)). Based on Figure 4.33 (b), the biosensor produced a stable signal baseline, within the range of -2  $\mu\text{A}$  to 3  $\mu\text{A}$  for a period of 7 days (21/5/2025 to 28/5/2025). By establishing a stable baseline at such a small monitoring range of 5  $\mu\text{A}$  (or 0.000005A sensitivity range), any minor anomalies above or below the threshold could be detected immediately.

Furthermore, the relatively fast transition between signal states indicates a short response time, which is essential for timely process adjustments in a dynamic treatment environment. These characteristics confirm that the MFC biosensor not only meets general sensitivity standards applied to chemical and biosensors but also offers robustness under real wastewater conditions.

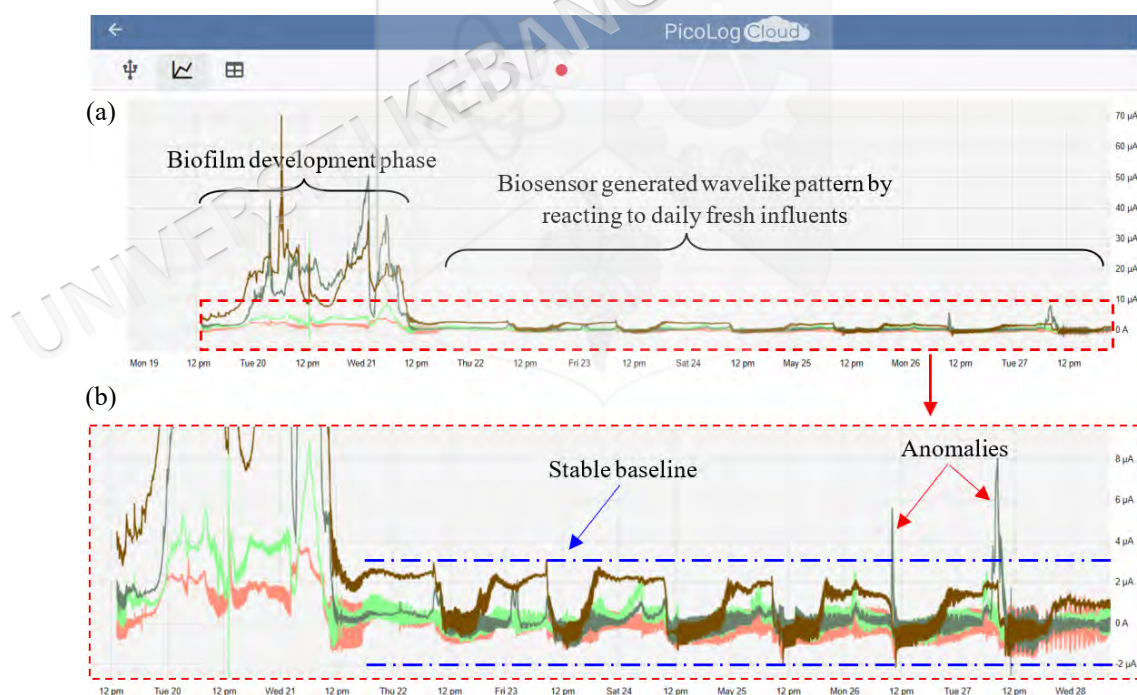


Figure 4.33

(a) Complete On-site data from biofilm development phase to stable phase and (b) biosensor's sensitivity range based on IWRC sewage tank.

## CHAPTER V

### CONCLUSION AND FUTURE DIRECTIONS

#### 5.1 CONCLUSION

In this research, a comprehensive approach was undertaken to address the limitations of conventional wastewater monitoring by developing a real-time bioelectrochemical sensor system integrated with an automated alarm notification platform. The study successfully demonstrated the potential of Microbial Electrochemical Sensors (MESe) and Microbial Fuel Cell Sensors (MFCSe) as sustainable, cost-effective, and rapid alternatives to traditional laboratory-based monitoring methods. The findings confirm the core hypotheses that electrode material selection and sensor design critically influence sensing performance, and that a robust Internet of Things (IoT)-integrated system can deliver reliable real-time data for practical wastewater applications.

The initial phase of the research focused on optimizing the MESe through surface modification of SS304L anodes. Among the carbon black-coated, flame oxidised, and acid bleached electrodes, the carbon black-coated MESe consistently exhibited the best performance, achieving the highest current density of up to  $1.3 \text{ A m}^{-2}$  and the strongest correlation with COD, with an  $R^2$  value reaching 0.9754 during thinner testing. This superior performance was attributed to the rough and porous carbon-coated surface, which enhanced microbial adhesion, promoted stable biofilm formation, and facilitated efficient extracellular electron transfer. In contrast, the acid bleached MESe produced the lowest current densities and weakest correlations, confirming its limited suitability for bioelectrochemical sensing. Despite its strong electrochemical performance, the MESe configuration exhibited signal instability during prolonged

operation, primarily due to clogging and mass transport limitations between the anode and cathode compartments.

To overcome these limitations, a novel tubular-type MFCSe with an air-cathode was designed and evaluated. This configuration eliminated the need for an external power supply, improved oxygen availability through direct air exposure, and significantly enhanced long-term operational stability. Among the different air-cathode formulations tested, the  $2.0 \text{ mg cm}^{-2}$  PTFE/CB/PtC-MFCSe emerged as the optimal design, delivering the highest and most stable current density of approximately  $0.31 \text{ A m}^{-2}$ . This sensor also demonstrated a strong linear relationship with COD, achieving an  $R^2$  value of 0.9655 during malathion testing, thereby confirming its reliability for detecting toxic wastewater contaminants. The improved performance of this MFCSe configuration highlights the importance of balanced catalyst loading, gas diffusion layer integrity, and electrode durability in sustaining bioelectrochemical activity.

A key contribution of this work is the successful integration of the MFCSe with an IoT-enabled monitoring platform incorporating mathematical models such as rate-of-change (RoC) analysis. This system enabled real-time signal processing and automated alarm notifications in response to sudden changes in wastewater composition. The long-term field deployment of the integrated MFCSe at a municipal wastewater treatment plant demonstrated stable signal output, reliable pollutant detection, and resilience under fluctuating operational conditions, providing strong evidence of its practical applicability.

In summary, this study delivers a holistic and scalable solution for real-time wastewater monitoring by advancing both sensor design and system-level integration. The carbon black-coated MESe was identified as the most effective configuration for high-sensitivity laboratory sensing, while the  $2.0 \text{ mg cm}^{-2}$  PtC-loaded tubular MFCSe proved to be the most robust and practical design for continuous, field-based monitoring. Together, these findings advance the field of bioelectrochemical sensing and establish a strong foundation for future work aimed at expanding pollutant detection ranges, improving sensor automation, and supporting intelligent, decentralized wastewater management systems.

Beyond the technical outcomes, this research contributes important practical insights into the deployment of bioelectrochemical sensors for real-world wastewater monitoring. By systematically evaluating sensor responses to biodegradable substrates, complex industrial effluents, and toxic pollutants, the study demonstrates how biofilm–electrode interactions govern signal stability and sensitivity under different contamination scenarios. The findings show that while high organic loading enhances current generation, toxic compounds suppress microbial activity and decouple electrochemical signals from conventional water quality indicators. This distinction is critical for interpreting sensor outputs in operational settings and underscores the need for sensor designs that are resilient to fluctuating wastewater compositions. The results further highlight the importance of long-term stability, ease of maintenance, and autonomous operation, all of which are essential for transitioning bioelectrochemical sensing technologies from laboratory-scale prototypes to practical monitoring tools in wastewater treatment infrastructure.

## **5.2 FUTURE DIRECTIONS**

While this study has demonstrated the feasibility and robustness of IoT-integrated MESe and MFCSe systems for real-time wastewater monitoring, further work is required to expand their applicability and performance. Future research should focus on enhancing sensor selectivity, extending long-term operational stability, and improving system intelligence to support wider deployment across diverse wastewater environments.

- a. Future studies should investigate the responses of MESe and MFCSe to a broader range of emerging contaminants, including pharmaceuticals, heavy metals, and mixed industrial effluents, to improve sensor versatility.
- b. Robust calibration models should be developed to quantitatively relate current density outputs to standard water quality parameters such as COD and BOD under varying operational conditions.

- c. Alternative electrode materials and catalyst formulations with reduced noble metal (Pt/C) loading should be explored to enhance cost-effectiveness without compromising sensor performance.
- d. Advanced data analytics and machine learning approaches should be integrated with IoT platforms to improve anomaly detection, trend analysis, and predictive maintenance capabilities.
- e. Long-term and multi-site field deployment studies should be conducted to evaluate sensor durability, maintenance requirements, and reliability under different wastewater treatment configurations.
- f. Scale-up studies should be performed to assess the feasibility of deploying multiple MFCSe units in parallel or modular configurations for monitoring large-volume wastewater systems and full-scale treatment facilities.



## REFERENCES

- Agrahari, R., Bayar, B., Abubackar, H. N., Giri, B. S., Rene, E. R., & Rani, R. 2022. Advances in the development of electrode materials for improving the reactor kinetics in microbial fuel cells. *Chemosphere* 290: 133184.
- Ahtasham Iqbal, M., Akram, S., khalid, S., Lal, B., Hassan, S. U., Ashraf, R., Kezembayeva, G., Mushtaq, M., Chinibayeva, N., & Hosseini-Bandegharai, A. 2024. Advanced photocatalysis as a viable and sustainable wastewater treatment process: A comprehensive review. *Environmental Research* 253: 118947.
- Al-Badani, M., Chong, P. L., & Lim, H. S. 2024. A mini review of the effect of modified carbon paper, carbon felt, and carbon cloth electrodes on the performance of microbial fuel cell. *International Journal of Green Energy* 21(1): 170–186.
- Al-Hameedi, A. T., Alkinani, H. H., Dunn-Norman, S., Alkhamis, M. M., Al-Alwani, M. A., Mutar, R. A., & Salem, E. 2020. Proposing a new biodegradable thinner and fluid loss control agent for water-based drilling fluid applications. *International Journal of Environmental Science and Technology* 17(8): 3621–3632.
- AlMetwally, S. A. H., Hassan, M. K., & Mourad, M. H. 2020. Real Time Internet of Things (IoT) Based Water Quality Management System. *Procedia CIRP* 91: 478–485.
- Altowayti, W. A., Shahir, S., Othman, N., Eisa, T. A., Yafooz, W. M. S., Al-Dhaqm, A., Soon, C. Y., Yahya, I. B., Che Rahim, N. A., Abaker, M., & Ali, A. 2022. The Role of Conventional Methods and Artificial Intelligence in the Wastewater Treatment: A Comprehensive Review. In *Processes* (Vol. 10).
- Andronie, M., Lăzăroiu, G., Iatagan, M., Uță, C., Ștefănescu, R., & Cocoșatu, M. 2021. Artificial Intelligence-Based Decision-Making Algorithms, Internet of Things Sensing Networks, and Deep Learning-Assisted Smart Process Management in Cyber-Physical Production Systems. In *Electronics* (Vol. 10).
- Astorga, S. E., Hu, L. X., Marsili, E., & Huang, Y. 2020. Ordered micropillar array gold electrode increases electrochemical signature of early biofilm attachment. *Materials & Design* 185: 108256.
- Bala, J. D., Lalung, J., & Ismail, N. 2015. Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. *International Journal of Recycling of Organic Waste in Agriculture* 4(1): 1–10.
- Balaram, V., Copia, L., Kumar, U. S., Miller, J., & Chidambaram, S. 2023. Pollution of water resources and application of ICP-MS techniques for monitoring and management—A comprehensive review. *Geosystems and Geoenvironment* 2(4): 100210.
- Ban, G.-H., Li, Y., Wall, M. M., & Jun, S. 2020. A Nanoengineered Stainless Steel Surface to Combat Bacterial Attachment and Biofilm Formation. In *Foods* (Vol. 9).
- Bankole, P. O., Semple, K. T., Jeon, B.-H., & Govindwar, S. P. 2021. Impact of redox-mediators in the degradation of olsalazine by marine-derived fungus,

- Aspergillus aculeatus* strain bpo2: Response surface methodology, laccase stability and kinetics. *Ecotoxicology and Environmental Safety* 208: 111742.
- Bretschger, O., Osterstock, J. B., Pinchak, W. E., Ishii, S. i., & Nelson, K. E. 2010. Microbial Fuel Cells and Microbial Ecology: Applications in Ruminant Health and Production Research. *Microbial Ecology* 59(3): 415–427.
- Buitrón, G., López-Prieto, I., Zúñiga, I. T., & Vargas, A. 2017. Reduction of start-up time in a microbial fuel cell through the variation of external resistance. *Energy Procedia* 142: 694–699.
- Butt, A., & Khan, M. X. 2022. Evaluation of Water Quality Using Physicochemical Parameters and Aquatic Insects Diversity. In R. Ray, D. G. Panagoulia, & N. Abeysingha (Eds.), *River Basin Management - Under a Changing Climate*.
- Campaña, A. L., Florez, S. L., Noguera, M. J., Fuentes, O. P., Ruiz Puentes, P., Cruz, J. C., & Osma, J. F. 2019. Enzyme-Based Electrochemical Biosensors for Microfluidic Platforms to Detect Pharmaceutical Residues in Wastewater. In *Biosensors* (Vol. 9).
- Cao, H., Wei, D., Yang, Y., Shang, Y., Li, G., Zhou, Y., Ma, Q., & Xu, Y. 2017. Systems-level understanding of ethanol-induced stresses and adaptation in *E. coli*. *Scientific Reports* 7(1): 44150.
- Cao, Z., Yan, W., Ding, M., & Yuan, Y. 2022. Construction of microbial consortia for microbial degradation of complex compounds. *Frontiers in Bioengineering and Biotechnology* 10.
- Cardoso Juarez, A. O., Ivan Ocampo Lopez, E., Kesarla, M. K., & Bogireddy, N. K. R. 2024. Advances in 4-Nitrophenol Detection and Reduction Methods and Mechanisms: An Updated Review. *ACS Omega* 9(31): 33335–33350.
- Champigneux, P., Renault-Sentenac, C., Bourrier, D., Rossi, C., Delia, M.-L., & Bergel, A. 2019. Effect of surface roughness, porosity and roughened micro-pillar structures on the early formation of microbial anodes. *Bioelectrochemistry* 128: 17–29.
- Chen, J.-J., Chen, W., He, H., Li, D.-B., Li, W.-W., Xiong, L., & Yu, H.-Q. 2013. Manipulation of Microbial Extracellular Electron Transfer by Changing Molecular Structure of Phenazine-Type Redox Mediators. *Environmental Science & Technology* 47(2): 1033–1039.
- Chen, L., Jiang, L., Cheng, L., Gao, Y., Wang, M., Xu, L., & Zhu, Z. 2024. Kinetic study of electron transfer process in methyl orange decolorization by shewanella in MFCs with covalent organic frameworks modified anode. *Chemosphere* 350: 141073.
- Chen, Q., Pu, W., Hou, H., Hu, J., Liu, B., Li, J., Cheng, K., Huang, L., Yuan, X., Yang, C., & Yang, J. 2018. Activated microporous-mesoporous carbon derived from chestnut shell as a sustainable anode material for high performance microbial fuel cells. *Bioresource Technology* 249: 567–573.
- Cheng, J., Xia, R., Li, H., Chen, Z., Zhou, X., Ren, X., Dong, H., Lin, R., & Zhou, J. 2022. Enhancing Extracellular Electron Transfer of *Geobacter sulfurreducens* in Bioelectrochemical Systems Using N-Doped Fe<sub>3</sub>O<sub>4</sub>@Carbon Dots. *ACS Sustainable Chemistry & Engineering* 10(12): 3935–3950.

- Choudhary, M., Verma, P., & Ray, S. 2024. A comprehensive review on bio-electrochemical systems for wastewater treatment: process, electricity generation and future aspect. *Environment, Development and Sustainability*.
- Chu, N., Liang, Q., Hao, W., Jiang, Y., & Zeng, R. J. 2021. Micro-microbial electrochemical sensor equipped with combined bioanode and biocathode for water biotoxicity monitoring. *Bioresource Technology* 326: 124743.
- Cui, Y., Lai, B., & Tang, X. 2019. Microbial Fuel Cell-Based Biosensors. In *Biosensors* (Vol. 9).
- Dai, Q., Zhang, S., Liu, H., Huang, J., & Li, L. 2020. Sulfide-mediated azo dye degradation and microbial community analysis in a single-chamber air cathode microbial fuel cell. *Bioelectrochemistry* 131: 107349.
- Debnath, K., & Dutta, S. 2023. Bio-electrochemical system analysis and improvement: A technical review. *Cleaner and Circular Bioeconomy* 6: 100052.
- Dubale, A. A., Su, W.-N., Tamirat, A. G., Pan, C.-J., Aragaw, B. A., Chen, H.-M., Chen, C.-H., & Hwang, B.-J. 2014. The synergetic effect of graphene on Cu<sub>2</sub>O nanowire arrays as a highly efficient hydrogen evolution photocathode in water splitting. *Journal of Materials Chemistry A* 2(43): 18383–18397.
- Edvardsen, L., Gawel, K., Wenner, S., Gawel, B., & Torsæter, M. 2020. Electrochemical enhancement and inhibition of calcium carbonate deposition. *Journal of Environmental Chemical Engineering* 8(5): 104239.
- Eifert, T., Eisen, K., Maiwald, M., & Herwig, C. 2020. Current and future requirements to industrial analytical infrastructure—part 2: smart sensors. *Analytical and Bioanalytical Chemistry* 412(9): 2037–2045.
- El-Sonbati, A. Z., Mohamed, G. G., El-Bindary, A. A., Hassan, W. M. I., Diab, M. A., Morgan, S. M., & Elkholy, A. K. 2015. Supramolecular structure, molecular docking and thermal properties of azo dye complexes. *Journal of Molecular Liquids* 212: 487–502.
- Emerson, D. 2018. The role of iron-oxidizing bacteria in biocorrosion: a review. *Biofouling* 34(9): 989–1000.
- Environment Quality (Industrial Effluent) Regulations 2009. *Department of Environment (DOE), Malaysia*: Retrieved December 12, 2025.
- Erbay, C., Pu, X., Choi, W., Choi, M.-J., Ryu, Y., Hou, H., Lin, F., de Figueiredo, P., Yu, C., & Han, A. 2015. Control of geometrical properties of carbon nanotube electrodes towards high-performance microbial fuel cells. *Journal of Power Sources* 280: 347–354.
- Erhan, L., Ndubuaku, M., Di Mauro, M., Song, W., Chen, M., Fortino, G., Bagdasar, O., & Liotta, A. 2021. Smart anomaly detection in sensor systems: A multi-perspective review. *Information Fusion* 67: 64–79.
- Esposito, M., Palma, L., Belli, A., Sabbatini, L., & Pierleoni, P. 2022. Recent Advances in Internet of Things Solutions for Early Warning Systems: A Review. In *Sensors* (Vol. 22).
- Estrada-Arriaga, E. B., Hernández-Romano, J., García-Sánchez, L., Guillén Garcés, R. A., Bahena-Bahena, E. O., Guadarrama-Pérez, O., & Moeller Chavez, G. E.

2018. Domestic wastewater treatment and power generation in continuous flow air-cathode stacked microbial fuel cell: Effect of series and parallel configuration. *Journal of Environmental Management* 214: 232–241.
- Exner, K. S. 2023. Importance of the volcano slope to comprehend activity and selectivity trends in electrocatalysis. *Current Opinion in Electrochemistry* 39: 101284.
- Fan, X., Zhou, Y., Jin, X., Song, R.-B., Li, Z., & Zhang, Q. 2021. Carbon material-based anodes in the microbial fuel cells. *Carbon Energy* 3(3): 449–472.
- Fang, Z., Hu, J., Xu, M.-Y., Li, S.-W., Li, C., Zhou, X., & Wei, J. 2024. A biocompatible electrode/exoelectrogens interface augments bidirectional electron transfer and bioelectrochemical reactions. *Bioelectrochemistry* 158: 108723.
- Fischer, F., Merino, N., Sugnaux, M., Huguenin, G., & Neilson, K. H. 2022. Microbial community diversity changes during voltage reversal repair in a 12-unit microbial fuel cell. *Chemical Engineering Journal* 446: 137334.
- Forhad, H. M., Uddin, M. R., Chakrovorty, R. S., Ruhul, A. M., Faruk, H. M., Kamruzzaman, S., Sharmin, N., Jamal, A. H. M. S. I. M., Haque, M. M.-U., & Morshed, A. M. 2024. IoT based real-time water quality monitoring system in water treatment plants (WTPs). *Heliyon* 10(23).
- Franza, T., & Gaudu, P. 2022. Quinones: more than electron shuttles. *Research in Microbiology* 173(6): 103953.
- Gandu, B., Rozenfeld, S., Ouaknin Hirsch, L., Schechter, A., & Cahan, R. 2020. Immobilization of bacterial cells on carbon-cloth anode using alginate for hydrogen generation in a microbial electrolysis cell. *Journal of Power Sources* 455: 227986.
- Garrido-Momparler, V., & Peris, M. 2022. Smart sensors in environmental/water quality monitoring using IoT and cloud services. *Trends in Environmental Analytical Chemistry* 35: e00173.
- Gemünde, A., Lai, B., Pause, L., Krömer, J., & Holtmann, D. 2022. Redox Mediators in Microbial Electrochemical Systems. *ChemElectroChem* 9(13): e202200216.
- Ghinea, L. M., Miron, M., & Barbu, M. 2023. Semi-Supervised Anomaly Detection of Dissolved Oxygen Sensor in Wastewater Treatment Plants. In *Sensors* (Vol. 23).
- Ghosh, N., Krushna, P. V., Sharma, J. D., & Srivastava, A. 2025. Predicting Blood/Air Partition Coefficients of Paint Thinner Ingredients and Assessing Their Inhalation Exposure Hazards. *ACS Chemical Health & Safety* 32(3): 297–306.
- Godain, A., Spurr, M. W. A., Boghani, H. C., Premier, G. C., Yu, E. H., & Head, I. M. 2020. Detection of 4-Nitrophenol, a Model Toxic Compound, Using Multi-Stage Microbial Fuel Cells. *Frontiers in Environmental Science* Volume 8 - 2020.
- Gong, Z., Yu, H., Zhang, J., Li, F., & Song, H. 2020. Microbial electro-fermentation for synthesis of chemicals and biofuels driven by bi-directional extracellular electron transfer. *Synthetic and Systems Biotechnology* 5(4): 304–313.
- González-Cortés, J. J., Lamprea-Pineda, P. A., Valle, A., Ramírez, M., Van Langenhove, H., Demeestere, K., & Walgraeve, C. 2024. Effect of toluene on siloxane

- biodegradation and microbial communities in biofilters. *Waste Management* 186: 119–129.
- González-Martínez, E., Saem, S., Beganovic, N. E., & Moran-Mirabal, J. M. 2023. Electrochemical Nano-Roughening of Gold Microstructured Electrodes for Enhanced Sensing in Biofluids. *Angewandte Chemie International Edition* 62(19): e202218080.
- Gralnick, J. A., & Bond, D. R. 2023. Electron Transfer Beyond the Outer Membrane: Putting Electrons to Rest. *Annual Review of Microbiology* 77(Volume 77, 2023): 517–539.
- Greenman, J., Gajda, I., You, J., Mendis, B. A., Obata, O., Pasternak, G., & Ieropoulos, I. 2021. Microbial fuel cells and their electrified biofilms. *Biofilm* 3: 100057.
- Greenman, J., Mendis, B. A., Gajda, I., & Ieropoulos, I. A. 2022. Microbial fuel cell compared to a chemostat. *Chemosphere* 296: 133967.
- Gulati, K., Kumar Boddu, R. S., Kapila, D., Bangare, S. L., Chandnani, N., & Saravanan, G. 2022. A review paper on wireless sensor network techniques in Internet of Things (IoT). *Materials Today: Proceedings* 51: 161–165.
- Guler, M., Turkoglu, V., & Kivrak, A. 2016. Electrochemical detection of malathion pesticide using acetylcholinesterase biosensor based on glassy carbon electrode modified with conducting polymer film. *Environmental Science and Pollution Research* 23(12): 12343–12351.
- Guo, K., Donose, B. C., Soeriyadi, A. H., PrévotEAU, A., Patil, S. A., Freguia, S., Gooding, J. J., & Rabaey, K. 2014. Flame Oxidation of Stainless Steel Felt Enhances Anodic Biofilm Formation and Current Output in Bioelectrochemical Systems. *Environmental Science & Technology* 48(12): 7151–7156.
- Guo, K., Soeriyadi, A. H., Feng, H., PrévotEAU, A., Patil, S. A., Gooding, J. J., & Rabaey, K. 2015. Heat-treated stainless steel felt as scalable anode material for bioelectrochemical systems. *Bioresource Technology* 195: 46–50.
- Guo, T., Wang, J., Yu, X., Jia, X., Zheng, X., Fang, Y., & Yang, Q. 2022. Improvement of microbial extracellular electron transfer via outer membrane cytochromes expression of engineered bacteria. *Biochemical Engineering Journal* 187: 108636.
- Guo, W., Ying, X., Zhao, N., Yu, S., Zhang, X., Feng, H., Zhang, Y., & Yu, H. 2023. Interspecies electron transfer between *Geobacter* and denitrifying bacteria for nitrogen removal in bioelectrochemical system. *Chemical Engineering Journal* 455: 139821.
- Güven, D., Hanhan, O., Aksoy, E. C., Insel, G., & Çokgör, E. 2017. Impact of paint shop decanter effluents on biological treatability of automotive industry wastewater. *Journal of Hazardous materials* 330: 61–67.
- Haghnegahdar, L., Joshi, S. S., & Dahotre, N. B. 2022. From IoT-based cloud manufacturing approach to intelligent additive manufacturing: industrial Internet of Things—an overview. *The International Journal of Advanced Manufacturing Technology* 119(3): 1461–1478.
- Halley, J. W., Schofield, A., & Berntson, B. 2012. Use of magnetite as anode for electrolysis of water. *Journal of Applied Physics* 111(12): 124911.

- Hasan, H. A., Rahim, N. F., Alias, J., Ahmad, J., Said, N. S., Ramli, N. N., Buhari, J., Abdullah, S. R., Othman, A. R., Jusoh, H. H., Juahir, H., & Kurniawan, S. B. 2024. A Review on the Roles of Extracellular Polymeric Substances (EPSs) in Wastewater Treatment: Source, Mechanism Study, Bioproducts, Limitations, and Future Challenges. In *Water* (Vol. 16, pp. 2812).
- Hasan, M. I., & Aggarwal, S. 2025. Matrix matters: How extracellular substances shape biofilm structure and mechanical properties. *Colloids and Surfaces B: Biointerfaces* 246: 114341.
- Hasson, D., Shemer, H., & Semiat, R. 2016. Removal of scale-forming ions by a novel cation-exchange electrochemical system—A review. *Desalination and Water Treatment* 57(48): 23147–23161.
- Ho, Q. N., Lau, W. J., Jaafar, J., Othman, M. H., & Yoshida, N. 2025. Membrane Technology for Valuable Resource Recovery from Palm Oil Mill Effluent (POME): A Review. In *Membranes* (Vol. 15, pp. 138).
- Horinouchi, T., Maeda, T., & Furusawa, C. 2018. Understanding and engineering alcohol-tolerant bacteria using OMICS technology. *World Journal of Microbiology and Biotechnology* 34(11): 157.
- Horst, A. E. W., Mangold, K.-M., & Holtmann, D. 2016. Application of gas diffusion electrodes in bioelectrochemical syntheses and energy conversion. *Biotechnology and Bioengineering* 113(2): 260–267.
- Hou, J., Liu, Z., Yang, S., & Zhou, Y. 2014. Three-dimensional macroporous anodes based on stainless steel fiber felt for high-performance microbial fuel cells. *Journal of Power Sources* 258: 204–209.
- Huang, L.-Z., Hansen, H. C. B., & Bjerrum, M. J. 2016. Electrochemical reduction of nitroaromatic compounds by single sheet iron oxide coated electrodes. *Journal of Hazardous materials* 306: 175–183.
- Huang, T., Liu, L., Tao, J., Zhou, L., & Zhang, S. 2018. Microbial fuel cells coupling with the three-dimensional electro-Fenton technique enhances the degradation of methyl orange in the wastewater. *Environmental Science and Pollution Research* 25(18): 17989–18000.
- Huang, Y., Liu, Y., Fan, K., & Xia, S. 2023. Influence of powdered activated carbon on gravity-driven ultrafiltration for decentralized drinking water treatment: Insights from microbial community and biofilm structure. *Separation and Purification Technology* 313: 123451.
- Hung, Y.-H., Liu, T.-Y., & Chen, H.-Y. 2019. Renewable Coffee Waste-Derived Porous Carbons as Anode Materials for High-Performance Sustainable Microbial Fuel Cells. *ACS Sustainable Chemistry & Engineering* 7(20): 16991–16999.
- Ibrahim, M. F., Hod, R., Toha, H. R., Mohammed Nawi, A., Idris, I. B., Mohd Yusoff, H., & Sahani, M. 2021. The Impacts of Illegal Toxic Waste Dumping on Children's Health: A Review and Case Study from Pasir Gudang, Malaysia. In *International Journal of Environmental Research and Public Health* (Vol. 18).
- Idris, A. O., Orimolade, B., Dennany, L., Mamba, B., Azizi, S., Kaviyarasu, K., & Maaza, M. 2023. A Review on Monitoring of Organic Pollutants in Wastewater Using Electrochemical Approach. *Electrocatalysis* 14(5): 659–687.

- Ismail, S. N. S., Abidin, E. Z., & Rasdi, I. 2020. A Case Study of Pasir Gudang Chemical Toxic Pollution: A Review on Health Symptoms, Psychological Manifestation and Biomarker Assessment *Malaysian Journal of Medicine and Health Sciences* 16: 175–184.
- Ito, H., Abe, K., Ishida, M., Hwang, C. M., & Nakano, A. 2015. Effect of through-plane polytetrafluoroethylene distribution in a gas diffusion layer on a polymer electrolyte unitized reversible fuel cell. *International Journal of Hydrogen Energy* 40(46): 16556–16565.
- Jadhav, D. A., Chendake, A. D., Pandit, S., Mungray, A. K., Jang, J.-H., Kumar, G., Al-Qaradawi, S. Y., Abdelkareem, M. A., & Chae, K.-J. 2025. Is the bioelectrochemical system ready for industrial commercialization? *Journal of Power Sources* 629: 236001.
- Jia, Y., Qian, D., Chen, Y., & Hu, Y. 2021. Intra/extracellular electron transfer for aerobic denitrification mediated by in-situ biosynthesis palladium nanoparticles. *Water Research* 189: 116612.
- Jiang, Y., & Zeng, R. J. 2019. Bidirectional extracellular electron transfers of electrode-biofilm: Mechanism and application. *Bioresource Technology* 271: 439–448.
- Jo, J., Jo, B., Kim, J., Kim, S., & Han, W. 2020. Development of an IoT-Based Indoor Air Quality Monitoring Platform. *Journal of Sensors* 2020(1): 8749764.
- Jukanti, A., Ebenezer, J., Chiliveru, A., Bommagani, V. K., Gandu, B., Schechter, A., & Cahan, R. 2026. Carbon black nanoparticles embedded in anodic biofilm to facilitate electron transfer in bio-electrochemical systems. *Chemical Engineering Journal* 528: 172378.
- Kacaribu, A. A., Aisyah, Y., Febriani, & Darwin. 2025. Development of wastewater treatment methods for palm oil mill effluent (POME): A comprehensive review. *Resources Chemicals and Materials* 4(4): 100130.
- Kadier, A., Wang, J., Chandrasekhar, K., Abdesahian, P., Islam, M. A., Ghanbari, F., Bajpai, M., Katoch, S. S., Bhagawati, P. B., Li, H., Kalil, M. S., Hamid, A. A., Abu Hasan, H., & Ma, P.-C. 2022. Performance optimization of microbial electrolysis cell (MEC) for palm oil mill effluent (POME) wastewater treatment and sustainable Bio-H<sub>2</sub> production using response surface methodology (RSM). *International Journal of Hydrogen Energy* 47(34): 15464–15479.
- Kaivonen, S., & Ngai, E. C. H. 2020. Real-time air pollution monitoring with sensors on city bus. *Digital Communications and Networks* 6(1): 23–30.
- Kalyani, N., Goel, S., & Jaiswal, S. 2021. On-site sensing of pesticides using point-of-care biosensors: a review. *Environmental Chemistry Letters* 19(1): 345–354.
- Kaneki, S., Kouketsu, Y., Aoya, M., Nakamura, Y., Wallis, S. R., Shimura, Y., & Yamaoka, K. 2024. An automatic peak deconvolution code for Raman spectra of carbonaceous material and a revised geothermometer for intermediate- to moderately high-grade metamorphism. *Progress in Earth and Planetary Science* 11(1): 35.

- Karlapudi, A. P., Venkateswarulu, T. C., Tammineedi, J., Kanumuri, L., Ravuru, B. K., Dirisala, V. r., & Kodali, V. P. 2018. Role of biosurfactants in bioremediation of oil pollution-a review. *Petroleum* 4(3): 241–249.
- Karn, A. L., Pandya, S., Mehbodniya, A., Arslan, F., Sharma, D. K., Phasinam, K., Aftab, M. N., Rajan, R., Bommiseti, R. K., & Sengan, S. 2023. An integrated approach for sustainable development of wastewater treatment and management system using IoT in smart cities. *Soft Computing* 27(8): 5159–5175.
- Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. 2020. Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. *Trends in Microbiology* 28(8): 668–681.
- Kataya, G., Issa, M., Badran, A., Cornu, D., Bechelany, M., Jellali, S., Jeguirim, M., & Hijazi, A. 2025. Dynamic removal of methylene blue and methyl orange from water using biochar derived from kitchen waste. *Scientific Reports* 15(1): 29907.
- Khan, A. U., Zahoor, M., Rehman, M. U., Shah, A. B., Zekker, I., Khan, F. A., Ullah, R., Albadrani, G. M., Bayram, R., & Mohamed, H. R. H. 2022. Biological Mineralization of Methyl Orange by *Pseudomonas aeruginosa*. In *Water* (Vol. 14, pp. 1551).
- Khan, M. D., Li, D., Tabraiz, S., Shamurad, B., Scott, K., Khan, M. Z., & Yu, E. H. 2021. Integrated air cathode microbial fuel cell-aerobic bioreactor set-up for enhanced bioelectrodegradation of azo dye Acid Blue 29. *Science of the Total Environment* 756: 143752.
- Khanal, S., Kc, K., Fulton, J. P., Shearer, S., & Ozkan, E. 2020. Remote Sensing in Agriculture—Accomplishments, Limitations, and Opportunities. In *Remote Sensing* (Vol. 12).
- Kim, B., Baek, G., Kim, C., Lee, S. Y., Yang, E., Lee, S., Kim, T., Nam, J.-Y., Lee, C., Chae, K.-J., Lee, H.-S., Park, H.-D., & Kim, J. R. 2024. Progress and Prospects for Applications of Extracellular Electron Transport Mechanism in Environmental Biotechnology. *ACS ES&T Engineering* 4(7): 1520–1539.
- Kishor, R., Purchase, D., Saratale, G. D., Romanholo Ferreira, L. F., Hussain, C. M., Mulla, S. I., & Bharagava, R. N. 2021. Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium *Pseudomonas aeruginosa* MZ520730. *Journal of Water Process Engineering* 43: 102300.
- Kobayashi, A., Fujii, T., Takeda, K., Tamoto, K., Kakinuma, K., & Uchida, M. 2022. Effect of Pt Loading Percentage on Carbon Blacks with Large Interior Nanopore Volume on the Performance and Durability of Polymer Electrolyte Fuel Cells. *ACS Applied Energy Materials* 5(1): 316–329.
- Koca, Y. O., Üstündağ, A. Ö., Yalcin, M., & Turgut, C. 2024. Assessing malathion residue impact on poultry health, human safety, and production performance. *Heliyon* 10(7).
- Koók, L., Nemestóthy, N., Bélafi-Bakó, K., & Bakonyi, P. 2021. The influential role of external electrical load in microbial fuel cells and related improvement strategies: A review. *Bioelectrochemistry* 140: 107749.

- Kralovec, C., & Schagerl, M. 2020. Review of Structural Health Monitoring Methods Regarding a Multi-Sensor Approach for Damage Assessment of Metal and Composite Structures. In *Sensors* (Vol. 20).
- Krishnamurthi, R., Kumar, A., Gopinathan, D., Nayyar, A., & Qureshi, B. 2020. An Overview of IoT Sensor Data Processing, Fusion, and Analysis Techniques. In *Sensors* (Vol. 20).
- Kwak, W.-J., Kim, H., Petit, Y. K., Leypold, C., Nguyen, T. T., Mahne, N., Redfern, P., Curtiss, L. A., Jung, H.-G., Borisov, S. M., Freunberger, S. A., & Sun, Y.-K. 2019. Deactivation of redox mediators in lithium-oxygen batteries by singlet oxygen. *Nature Communications* 10(1): 1380.
- Lambertz, S., Franke, M., Stelter, M., & Braeutigam, P. 2024. Determination of Chemical Oxygen Demand with electrochemical methods: A review. *Chemical Engineering Journal Advances* 18: 100615.
- Ledezma, P., Donose, B. C., Freguia, S., & Keller, J. 2015. Oxidised stainless steel: a very effective electrode material for microbial fuel cell bioanodes but at high risk of corrosion. *Electrochimica Acta* 158: 356–360.
- Li, B., Cao, S., Qin, Y., Liu, X., Xu, X., & Xin, Q. 2024. Liquid Water Transport and Distribution in the Gas Diffusion Layer of a Proton Exchange Membrane Fuel Cell Considering Interfacial Cracks. In *Energies* (Vol. 17, pp. 5339).
- Li, H., Yang, Q., Li, J., Gao, H., Li, P., & Zhou, H. 2015. The impact of temperature on microbial diversity and AOA activity in the Tengchong Geothermal Field, China. *Scientific Reports* 5(1): 17056.
- Li, J., Dong, Y., Hu, L., Zhang, Y., Fu, Q., Zhang, L., Zhu, X., & Liao, Q. 2022. Microalgae hydrogel-derived monolithic free-standing air cathode for microbial fuel cells: Tailoring the macroporous structure for enhanced bioelectricity generation. *Renewable and Sustainable Energy Reviews* 153: 111773.
- Li, J., Li, H., Fu, Q., Liao, Q., Zhu, X., Kobayashi, H., & Ye, D. 2017. Voltage reversal causes bioanode corrosion in microbial fuel cell stacks. *International Journal of Hydrogen Energy* 42(45): 27649–27656.
- Li, J., Song, B., Yao, C., Zhang, Z., Wang, L., & Zhang, J. 2022. S-Doped NiFe<sub>2</sub>O<sub>4</sub> Nanosheets Regulated Microbial Community of Suspension for Constructing High Electroactive Consortia. In *Nanomaterials* (Vol. 12).
- Li, L., Breedveld, V., & Hess, D. W. 2012. Creation of Superhydrophobic Stainless Steel Surfaces by Acid Treatments and Hydrophobic Film Deposition. *ACS Applied Materials & Interfaces* 4(9): 4549–4556.
- Li, M., Li, Y.-W., Cai, Q.-Y., Zhou, S.-Q., & Mo, C.-H. 2020. Spraying carbon powder derived from mango wood biomass as high-performance anode in bio-electrochemical system. *Bioresource Technology* 300: 122623.
- Li, Z., Luan, Y., Qu, Y., & Jing, L. 2015. Modification Strategies with Inorganic Acids for Efficient Photocatalysts by Promoting the Adsorption of O<sub>2</sub>. *ACS Applied Materials & Interfaces* 7(41): 22727–22740.
- Liang, G., Liu, Y., Gu, Z., Chen, X., Song, W., Wei, W., Wu, J., Hu, G., Zhao, J., Liu, L., & Gao, C. 2024. Shortening electron transfer distance to enhance chemicals

- and electric energy production in *Escherichia coli*. *Chemical Engineering Journal* 497: 154932.
- Lin, Z., Cheng, S., Sun, Y., Li, H., & Jin, B. 2022. Realizing BOD detection of real wastewater by considering the bioelectrochemical degradability of organic pollutants in a bioelectrochemical system. *Chemical Engineering Journal* 444: 136520.
- Liu, C., Yu, H., Zhang, B., Liu, S., Liu, C.-g., Li, F., & Song, H. 2022. Engineering whole-cell microbial biosensors: Design principles and applications in monitoring and treatment of heavy metals and organic pollutants. *Biotechnology Advances* 60: 108019.
- Liu, S.-H., Wu, C.-H., & Lin, C.-W. 2017. Enhancement of bioelectricity generation for an air-cathode microbial fuel cell using polyvinyl alcohol-membrane electrode assemblies. *Biochemical Engineering Journal* 128: 210–217.
- Liu, S., Li, Z., Liang, D., Yan, C., He, W., & Feng, Y. 2023. A novel self-bonding 3D carbon particle bioanode derived from agricultural residue for improving the enrichment of electroactive bacteria in microbial fuel cell. *Chemical Engineering Journal* 473: 145443.
- Liu, X., Wang, Y., Cong, H., Shen, Y., & Yu, B. 2021. A review of the design of packing materials for ion chromatography. *Journal of Chromatography A* 1653: 462313.
- Logan, B. E., Rossi, R., Ragab, A. a., & Saikaly, P. E. 2019. Electroactive microorganisms in bioelectrochemical systems. *Nature Reviews Microbiology* 17(5): 307–319.
- López Zavala, M. Á., & Cámara Gutiérrez, I. C. 2023. Effects of External Resistance, New Electrode Material, and Catholyte Type on the Energy Generation and Performance of Dual-Chamber Microbial Fuel Cells. In *Fermentation* (Vol. 9, pp. 344).
- Lu, R., Chen, Y., Wu, J., Chen, D., Wu, Z., & Xiao, E. 2022. In situ COD monitoring with use of a hybrid of constructed wetland-microbial fuel cell. *Water Research* 210: 117957.
- Lu, Z., & Imlay, J. A. 2021. When anaerobes encounter oxygen: mechanisms of oxygen toxicity, tolerance and defence. *Nature Reviews Microbiology* 19(12): 774–785.
- Luan, X., Liu, X., Fang, C., Chu, W., & Xu, Z. 2020. Ecotoxicological effects of disinfected wastewater effluents: a short review of in vivo toxicity bioassays on aquatic organisms. *Environmental Science: Water Research & Technology* 6(9): 2275–2286.
- Ma, J., Gu, Y., & Xu, P. 2020. A roadmap to engineering antiviral natural products synthesis in microbes. *Current Opinion in Biotechnology* 66: 140–149.
- Ma, Y., Deng, D., Zhan, Y., Cao, L., & Liu, Y. 2022. A systematic study on self-powered microbial fuel cell based BOD biosensors running under different temperatures. *Biochemical Engineering Journal* 180: 108372.
- Ma, Y., Wang, H., Sun, L., Liu, E., Fei, G., Fan, J., & Kang, Y.-M. 2022. Unidirectional electron transport from graphitic-C<sub>3</sub>N<sub>4</sub> for novel remote and long-term photocatalytic anti-corrosion on Q235 carbon steel. *Chemical Engineering Journal* 429: 132520.

- Mahto, K. U., Vandana, Priyadarshane, M., Samantaray, D. P., & Das, S. 2022. Bacterial biofilm and extracellular polymeric substances in the treatment of environmental pollutants: Beyond the protective role in survivability. *Journal of Cleaner Production* 379: 134759.
- Martí, L., Sanchez-Pi, N., Molina, J. M., & Garcia, A. C. 2015. Anomaly Detection Based on Sensor Data in Petroleum Industry Applications. In *Sensors* (Vol. 15, pp. 2774–2797).
- Matesun, J., Petrik, L., Musvoto, E., Ayinde, W., & Ikumi, D. 2024. Limitations of wastewater treatment plants in removing trace anthropogenic biomarkers and future directions: A review. *Ecotoxicology and Environmental Safety* 281: 116610.
- Matheri, A. N., Mohamed, B., Ntuli, F., Nabadda, E., & Ngila, J. C. 2022. Sustainable circularity and intelligent data-driven operations and control of the wastewater treatment plant. *Physics and Chemistry of the Earth, Parts A/B/C* 126: 103152.
- McCann, R., Obeidi, M. A., Hughes, C., McCarthy, É., Egan, D. S., Vijayaraghavan, R. K., Joshi, A. M., Acinas Garzon, V., Dowling, D. P., McNally, P. J., & Brabazon, D. 2021. In-situ sensing, process monitoring and machine control in Laser Powder Bed Fusion: A review. *Additive Manufacturing* 45: 102058.
- Md Khudzari, J., Gariépy, Y., Kurian, J., Tartakovsky, B., & Raghavan, G. S. V. 2019. Effects of biochar anodes in rice plant microbial fuel cells on the production of bioelectricity, biomass, and methane. *Biochemical Engineering Journal* 141: 190–199.
- Mejía-López, M., Lastres, O., Alemán-Ramirez, J. L., Lobato-Peralta, D. R., Verde, A., Gámez, J. J. M., de Paz, P. L., & Vereá, L. 2023. Conductive carbon-polymer composite for bioelectrodes and electricity generation in a sedimentary microbial fuel cell. *Biochemical Engineering Journal* 193: 108856.
- Merino-Jimenez, I., Obata, O., Pasternak, G., Gajda, I., Greenman, J., & Ieropoulos, I. 2021. Effect of microbial fuel cell operation time on the disinfection efficacy of electrochemically synthesised catholyte from urine. *Process Biochemistry* 101: 294–303.
- Militino, A. F., Moradi, M., & Ugarte, M. D. 2020. On the Performances of Trend and Change-Point Detection Methods for Remote Sensing Data. In *Remote Sensing* (Vol. 12).
- Mittal, Y., Dash, S., Srivastava, P., Mishra, P. M., Aminabhavi, T. M., & Yadav, A. K. 2022. Azo dye containing wastewater treatment in earthen membrane based unplanted two chambered constructed wetlands-microbial fuel cells: A new design for enhanced performance. *Chemical Engineering Journal* 427: 131856.
- Mohapatra, L., Paramanik, L., Sabnam, S., & Yoo, S. H. 2024. Advanced strategies for controlling three-phase boundaries in photocatalysis. *Nanoscale* 16(48): 22099–22119.
- Mohd Noor, N. N., Misali, R., & Kim, K. 2024. PTFE-based hydrophobic layers influence bioelectrical performance of sediment microbial fuel cells with floating air-cathode. *International Journal of Hydrogen Energy* 87: 469–475.

- Molderez, T. R., PrévotEAU, A., Ceyssens, F., Verhelst, M., & Rabaey, K. 2021. A chip-based 128-channel potentiostat for high-throughput studies of bioelectrochemical systems: Optimal electrode potentials for anodic biofilms. *Biosensors and Bioelectronics* 174: 112813.
- Mollaei, M., Timmers, P. H. A., Suarez-Diez, M., Boeren, S., van Gelder, A. H., Stams, A. J. M., & Plugge, C. M. 2021. Comparative proteomics of *Geobacter sulfurreducens* PCAT in response to acetate, formate and/or hydrogen as electron donor. *Environmental Microbiology* 23(1): 299–315.
- Moretti, A., Ivan, H. L., & Skvaril, J. 2024. A review of the state-of-the-art wastewater quality characterization and measurement technologies. Is the shift to real-time monitoring nowadays feasible? *Journal of Water Process Engineering* 60: 105061.
- Mu, M., Liu, S., DeFlorio, W., Hao, L., Wang, X., Salazar, K. S., Taylor, M., Castillo, A., Cisneros-Zevallos, L., Oh, J. K., Min, Y., & Akbulut, M. 2023. Influence of Surface Roughness, Nanostructure, and Wetting on Bacterial Adhesion. *Langmuir* 39(15): 5426–5439.
- Mukimin, A., & Vistanty, H. 2023. Low carbon development based on microbial fuel cells as electrical generation and wastewater treatment unit. *Renewable Energy Focus* 44: 132–138.
- Munoz-Cupa, C., & Bassi, A. 2024. Investigation of heavy metal removal from salty wastewater and voltage production using *Shewanella oneidensis* MR-1 nanowires in a dual-chamber microbial fuel cell. *Environmental Progress & Sustainable Energy* 43(1): e14237.
- Nair K, S., Manu, B., & Azhoni, A. 2021. Sustainable treatment of paint industry wastewater: Current techniques and challenges. *Journal of Environmental Management* 296: 113105.
- Navato, A. P., & Mueller, A. V. 2021. Enabling Automatic Detection of Anomalies in Wastewater: A Highly Simplified Approach to Defining “Normal” in Complex Chemical Mixtures. *Frontiers in Water* Volume 3 - 2021.
- Neale, P. A., Escher, B. I., de Baat, M. L., Dechesne, M., Dingemans, M. M. L., Enault, J., Pronk, G. J., Smeets, P. W. M. H., & Leusch, F. D. L. 2023. Application of Effect-Based Methods to Water Quality Monitoring: Answering Frequently Asked Questions by Water Quality Managers, Regulators, and Policy Makers. *Environmental Science & Technology* 57(15): 6023–6032.
- Obata, O., Salar-Garcia, M. J., Greenman, J., Kurt, H., Chandran, K., & Ieropoulos, I. 2020. Development of efficient electroactive biofilm in urine-fed microbial fuel cell cascades for bioelectricity generation. *Journal of Environmental Management* 258: 109992.
- Ojha, R., Dash, J., Satpathy, S. S., Ojha, P. C., & Pradhan, D. 2025. A brief review on factors affecting the performance of microbial fuel cell and integration of artificial intelligence. *Discover Sustainability* 6(1): 702.
- Oliveira, P., Salomé Duarte, M., & Novais, P. 2023. Conception and evaluation of anomaly detection models for monitoring analytical parameters in wastewater treatment plants. *AI Communications* 37(3): 443–465.

- Omeje, K. O., Ezema, B. O., Okonkwo, F., Onyishi, N. C., Ozioko, J., Rasag, W. A., Sardo, G., & Okpala, C. O. R. 2021. Quantification of Heavy Metals and Pesticide Residues in Widely Consumed Nigerian Food Crops Using Atomic Absorption Spectroscopy (AAS) and Gas Chromatography (GC). In *Toxins* (Vol. 13).
- Pan, Q., Guo, D., Gao, X., Heere, M., Lu, W., Vietor, T., & Gao, Y. 2024. Modulating triple phase boundary of oxygen reduction reaction catalyst for fast activation of proton exchange membrane fuel cell. *International Journal of Hydrogen Energy* 87: 409–430.
- Peng, X., Chen, S., Liu, L., Zheng, S., & Li, M. 2016. Modified stainless steel for high performance and stable anode in microbial fuel cells. *Electrochimica Acta* 194: 246–252.
- Perchikov, R., Cheliukanov, M., Plekhanova, Y., Tarasov, S., Kharkova, A., Butusov, D., Arlyapov, V., Nakamura, H., & Reshetilov, A. 2024. Microbial Biofilms: Features of Formation and Potential for Use in Bioelectrochemical Devices. In *Biosensors* (Vol. 14, pp. 302).
- Perelonia, K. B. S., Benitez, K. C. D., Banicod, R. J. S., Tadifa, G. C., Cambia, F. D., & Montojo, U. M. 2021. Validation of an analytical method for the determination of cadmium, lead and mercury in fish and fishery resources by graphite furnace and Cold Vapor Atomic Absorption Spectrometry. *Food Control* 130: 108363.
- Pierra, M., Golozar, M., Zhang, X., PrévotEAU, A., De Volder, M., Reynaerts, D., & Rabaey, K. 2018. Growth and current production of mixed culture anodic biofilms remain unaffected by sub-microscale surface roughness. *Bioelectrochemistry* 122: 213–220.
- Pinto, R. P., Srinivasan, B., Guiot, S. R., & Tartakovsky, B. 2011. The effect of real-time external resistance optimization on microbial fuel cell performance. *Water Research* 45(4): 1571–1578.
- Pluciennik-Koropczuk, E., & Myszograj, S. 2020. Changes in quantity of non-biodegradable organic micropollutants in municipal wastewater based on COD fractionation. *Desalination and Water Treatment* 186: 334–340.
- Poornima, I. G. A., & Paramasivan, B. 2020. Anomaly detection in wireless sensor network using machine learning algorithm. *Computer Communications* 151: 331–337.
- Priyadarshi, R. 2024. Energy-Efficient Routing in Wireless Sensor Networks: A Meta-heuristic and Artificial Intelligence-based Approach: A Comprehensive Review. *Archives of Computational Methods in Engineering* 31(4): 2109–2137.
- Priyanka, E. B., Thangavel, S., & Gao, X.-Z. 2021. Review analysis on cloud computing based smart grid technology in the oil pipeline sensor network system. *Petroleum Research* 6(1): 77–90.
- Pu, K.-B., Ma, Q., Cai, W.-F., Chen, Q.-Y., Wang, Y.-H., & Li, F.-J. 2018. Polypyrrole modified stainless steel as high performance anode of microbial fuel cell. *Biochemical Engineering Journal* 132: 255–261.

- Qinxia, H., Nazir, S., Li, M., Ullah Khan, H., Lianlian, W., & Ahmad, S. 2021. AI-Enabled Sensing and Decision-Making for IoT Systems. *Complexity* 2021(1): 6616279.
- Qiu, S., Guo, Z., Naz, F., Yang, Z., & Yu, C. 2021. An overview in the development of cathode materials for the improvement in power generation of microbial fuel cells. *Bioelectrochemistry* 141: 107834.
- Qiu, S., Wang, L., Zhang, Y., & Yu, Y. 2022. Microbial Fuel Cell-Based Biosensor for Simultaneous Test of Sodium Acetate and Glucose in a Mixed Solution. In *International Journal of Environmental Research and Public Health* (Vol. 19, pp. 12297).
- Quan, X.-c., Quan, Y.-p., & Tao, K. 2012. Effect of anode aeration on the performance and microbial community of an air-cathode microbial fuel cell. *Chemical Engineering Journal* 210: 150–156.
- Ramanaiah, S. V., Cordas, C. M., Matias, S. C., Reddy, M. V., Leitão, J. H., & Fonseca, L. P. 2021. Bioelectricity generation using long-term operated biocathode: RFLP based microbial diversity analysis. *Biotechnology Reports* 32: e00693.
- Ratheesh, A., Elias, L., & Aboobakar Shibli, S. M. 2021. Tuning of Electrode Surface for Enhanced Bacterial Adhesion and Reactions: A Review on Recent Approaches. *ACS Applied Bio Materials* 4(8): 5809–5838.
- Rauglas, E., Martin, S., Bailey, K., Magnuson, M., Phillips, R., & Harper, W. F. 2016. The effect of malathion on the activity, performance, and microbial ecology of activated sludge. *Journal of Environmental Management* 183: 220–228.
- Rehman, F. U., Amjad, F., Lee, W., & Chang, M. 2025. Recent advances in functional energy materials for microbial fuel cells: progress, challenges, and future perspectives. *Journal of Materials Chemistry A* 13(38): 32056–32103.
- Rezaei, A., Karami, Z., Feli, F., & Aber, S. 2023. Oxygen reduction reaction enhancement in microbial fuel cell cathode using cesium phosphomolybdate electrocatalyst. *Fuel* 352: 129040.
- Ribeiro, A. K. C., de Freitas, R. F. C. P., de Carvalho, I. H. G., de Miranda, L. M., da Silva, N. R., de Fátima Dantas de Almeida, L., Zhang, Y., da Fonte Porto Carreiro, A., & de Assunção e Souza, R. O. 2023. Flexural strength, surface roughness, micro-CT analysis, and microbiological adhesion of a 3D-printed temporary crown material. *Clinical Oral Investigations* 27(5): 2207–2220.
- Rodriguez-Caballero, A., Ramond, J. B., Welz, P. J., Cowan, D. A., Odlare, M., & Burton, S. G. 2012. Treatment of high ethanol concentration wastewater by biological sand filters: Enhanced COD removal and bacterial community dynamics. *Journal of Environmental Management* 109: 54–60.
- Romain Njimou, J., Tamwa Sieugaing, M., Péguy Nanseu-Njiki, C., & Ngameni, E. 2024. Bio-based Nanomaterial in Carefully Designed Carbon Paste Electrode for the Detection of Methyl Orange. *Electroanalysis* 36(6): e202300364.
- Rong, C., Huang, X., Arandiyani, H., Shao, Z., Wang, Y., & Chen, Y. 2025. Advances in Oxygen Evolution Reaction Electrocatalysts via Direct Oxygen–Oxygen Radical Coupling Pathway. *Advanced Materials* 37(9): 2416362.

- Sá, M. F. T., Castro, V., Gomes, A. I., Morais, D. F. S., Silva Braga, R. V. P. S., Saraiva, I., Souza-Chaves, B. M., Park, M., Fernández-Fernández, V., Rodil, R., Montes, R., Quintana, J. B., & Vilar, V. J. P. 2022. Tracking pollutants in a municipal sewage network impairing the operation of a wastewater treatment plant. *Science of the Total Environment* 817: 152518.
- Saadi, M., Pézard, J., Haddour, N., Erouel, M., Vogel, T. M., & Khirouni, K. 2020. Stainless steel coated with carbon nanofiber/PDMS composite as anodes in microbial fuel cells. *Materials Research Express* 7(2): 025504.
- Sagan, V., Peterson, K. T., Maimaitijiang, M., Sidike, P., Sloan, J., Greeling, B. A., Maalouf, S., & Adams, C. 2020. Monitoring inland water quality using remote sensing: potential and limitations of spectral indices, bio-optical simulations, machine learning, and cloud computing. *Earth-Science Reviews* 205: 103187.
- Salvian, A., Farkas, D., Ramírez-Moreno, M., Avignone Rossa, C., Varcoe, J. R., & Gadkari, S. 2024. Impact of Air-Cathodes on Operational Stability of Single-Chamber Microbial Fuel Cell Biosensors for Wastewater Monitoring. In *Energies* (Vol. 17).
- Samavati, Z., Goh, P. S., Fauzi Ismail, A., Lau, W. J., Samavati, A., Ng, B. C., & Sohaimi Abdullah, M. 2025. Advancements in membrane technology for efficient POME treatment: A comprehensive review and future perspectives. *Journal of Environmental Sciences* 155: 730–761.
- Sangamnere, R., Misra, T., Bherwani, H., Kapley, A., & Kumar, R. 2023. A critical review of conventional and emerging wastewater treatment technologies. *Sustainable Water Resources Management* 9(2): 58.
- Santini, M., Marzorati, S., Fest-Santini, S., Trasatti, S., & Cristiani, P. 2017. Carbonate scale deactivating the biocathode in a microbial fuel cell. *Journal of Power Sources* 356: 400–407.
- Santoro, C., Guilizzoni, M., Correa Baena, J. P., Pasaogullari, U., Casalegno, A., Li, B., Babanova, S., Artyushkova, K., & Atanassov, P. 2014. The effects of carbon electrode surface properties on bacteria attachment and start up time of microbial fuel cells. *Carbon* 67: 128–139.
- Sarkar, P., Maji, M., Ghosh, M., & Dey, A. 2022. Kinetic modelling of high concentration 4-Nitrophenol biodegradation by an isolated bacterial consortium and post-treatment ecotoxicity analysis. *Biocatalysis and Agricultural Biotechnology* 41: 102316.
- Schiessl, K. T., Hu, F., Jo, J., Nazia, S. Z., Wang, B., Price-Whelan, A., Min, W., & Dietrich, L. E. P. 2019. Phenazine production promotes antibiotic tolerance and metabolic heterogeneity in *Pseudomonas aeruginosa* biofilms. *Nature Communications* 10(1): 762.
- Sean, W.-Y., Chu, Y.-Y., Mallu, L. L., Chen, J.-G., & Liu, H.-Y. 2020. Energy consumption analysis in wastewater treatment plants using simulation and SCADA system: Case study in northern Taiwan. *Journal of Cleaner Production* 276: 124248.

- Senna, M., Šepelák, V., Shi, J., Bauer, B., Feldhoff, A., Laporte, V., & Becker, K.-D. 2012. Introduction of oxygen vacancies and fluorine into TiO<sub>2</sub> nanoparticles by co-milling with PTFE. *Journal of Solid State Chemistry* 187: 51–57.
- Senthilkumar, K., & Naveenkumar, M. 2023. Enhanced performance study of microbial fuel cell using waste biomass-derived carbon electrode. *Biomass Conversion and Biorefinery* 13(7): 5921–5929.
- Sevda, S., Garlapati, V. K., Naha, S., Sharma, M., Ray, S. G., Sreekrishnan, T. R., & Goswami, P. 2020. Biosensing capabilities of bioelectrochemical systems towards sustainable water streams: Technological implications and future prospects. *Journal of Bioscience and Bioengineering* 129(6): 647–656.
- Seviour, T., Derlon, N., Dueholm, M. S., Flemming, H.-C., Girbal-Neuhauser, E., Horn, H., Kjelleberg, S., van Loosdrecht, M. C. M., Lotti, T., Malpei, M. F., Nerenberg, R., Neu, T. R., Paul, E., Yu, H., & Lin, Y. 2019. Extracellular polymeric substances of biofilms: Suffering from an identity crisis. *Water Research* 151: 1–7.
- Shah, A., Arjunan, A., Baroutaji, A., & Zakharova, J. 2023. A review of physicochemical and biological contaminants in drinking water and their impacts on human health. *Water Science and Engineering* 16(4): 333–344.
- Shamshad, J., & Ur Rehman, R. 2025. Innovative approaches to sustainable wastewater treatment: a comprehensive exploration of conventional and emerging technologies. *Environmental Science: Advances* 4(2): 189–222.
- Shanbhag, M. M., Manasa, G., Mascarenhas, R. J., Mondal, K., & Shetti, N. P. 2023. Fundamentals of bio-electrochemical sensing. *Chemical Engineering Journal Advances* 16: 100516.
- Sharma, M., Salama, E.-S., Thakur, N., Alghamdi, H., Jeon, B.-H., & Li, X. 2023. Advances in the biomass valorization in bioelectrochemical systems: A sustainable approach for microbial-aided electricity and hydrogen production. *Chemical Engineering Journal* 465: 142546.
- Simoska, O., Gaffney, E. M., Minter, S. D., Franzetti, A., Cristiani, P., Grattieri, M., & Santoro, C. 2021. Recent trends and advances in microbial electrochemical sensing technologies: An overview. *Current Opinion in Electrochemistry* 30: 100762.
- Singh, S., Singh, J., & Singh, H. 2021. Chapter 3 - Chemical oxygen demand and biochemical oxygen demand. In Inamuddin, R. Boddula, & A. M. Asiri (Eds.), *Green Sustainable Process for Chemical and Environmental Engineering and Science* (pp. 69–83).
- Sonawane, J. M., Ezugwu, C. I., & Ghosh, P. C. 2020. Microbial Fuel Cell-Based Biological Oxygen Demand Sensors for Monitoring Wastewater: State-of-the-Art and Practical Applications. *ACS Sensors* 5(8): 2297–2316.
- Song, Y.-C., Kim, D.-S., Woo, J.-H., Subha, B., Jang, S.-H., & Sivakumar, S. 2015. Effect of surface modification of anode with surfactant on the performance of microbial fuel cell. *International Journal of Energy Research* 39(6): 860–868.
- Song, Y., Gu, P., Liu, J., Sun, H., Cai, Z., Li, J., Xu, Z., Li, Z., & Zou, J. 2024. Navigating the developments of air-cathode catalysts for efficient and sustainable bio-

- energy production from wastewater in microbial fuel cells. *Coordination Chemistry Reviews* 517: 216019.
- Srivastava, P., Mittal, Y., Gupta, S., Abbassi, R., & Garaniya, V. 2022. Chapter 11 - Recent progress in biosensors for wastewater monitoring and surveillance. In M. Asadnia, A. Razmjou, & A. Beheshti (Eds.), *Artificial Intelligence and Data Science in Environmental Sensing* (pp. 245–267).
- Stratton, S. M., Zhang, S., & Montemore, M. M. 2023. Addressing complexity in catalyst design: From volcanos and scaling to more sophisticated design strategies. *Surface Science Reports* 78(3): 100597.
- Subhadra, B. 2022. Special Issue: Biofilm Composition and Applications. In *Coatings* (Vol. 12, pp. 1026).
- Suhane, S., Ali, K., Singh, P., & Asati, V. 2026. Advanced Analytical Techniques for Detection of Malathion Pesticide: A Comprehensive Review Based on Environmental Concerns. *SEPARATION SCIENCE PLUS* 9(1): e70187.
- Sun, M., Zhai, L.-F., Mu, Y., & Yu, H.-Q. 2020. Bioelectrochemical element conversion reactions towards generation of energy and value-added chemicals. *Progress in Energy and Combustion Science* 77: 100814.
- Syrmos, E., Sidiropoulos, V., Bechtsis, D., Stergiopoulos, F., Aivazidou, E., Vrakas, D., Vezinias, P., & Vlahavas, I. 2023. An Intelligent Modular Water Monitoring IoT System for Real-Time Quantitative and Qualitative Measurements. In *Sustainability* (Vol. 15).
- Tanveer, S., Ilyas, N., Akhtar, N., Akhtar, N., Bostan, N., Hasnain, Z., Niaz, A., Zengin, G., Gafur, A., & Fitriatin, B. N. 2024. Unlocking the interaction of organophosphorus pesticide residues with ecosystem: Toxicity and bioremediation. *Environmental Research* 249: 118291.
- Thengumthottathil, V., Ponnusamy, K., & Naina Mohamed, S. 2024. Bioelectrochemical systems: Exploring microbial communities, interactions, and electron transfer. *Biochemical Engineering Journal* 211: 109442.
- Tian, X., Yin, X., Wang, Z., Ren, Z., Liu, H., Li, S., & Bian, D. 2024. Effect of lowering temperature on the function and interactions of microbial communities in micro pressure swirl reactor under low-carbon source conditions. *Journal of Water Process Engineering* 58: 104845.
- Varjani, S. 2022. Prospective review on bioelectrochemical systems for wastewater treatment: Achievements, hindrances and role in sustainable environment. *Science of the Total Environment* 841: 156691.
- Velusamy, K., Periyasamy, S., Kumar, P. S., Rangasamy, G., Nisha Pauline, J. M., Ramaraju, P., Mohanasundaram, S., & Nguyen Vo, D.-V. 2022. Biosensor for heavy metals detection in wastewater: A review. *Food and Chemical Toxicology* 168: 113307.
- Verma, M., Singh, V., & Mishra, V. 2023. Moving towards the enhancement of extracellular electron transfer in electrogens. *World Journal of Microbiology and Biotechnology* 39(5): 130.
- Verma, M. L., & Rani, V. 2021. Biosensors for toxic metals, polychlorinated biphenyls, biological oxygen demand, endocrine disruptors, hormones, dioxin, phenolic

- and organophosphorus compounds: a review. *Environmental Chemistry Letters* 19(2): 1657–1666.
- Vignesh, S., Rajkumar, C., Singh Gautam, R. K., Suganthi, S., Ahmad, K., & Oh, T. H. 2026. Recent Progress in Advanced Electrode Materials for the Detection of 4-Nitrophenol and Its Derivatives for Environmental Monitoring. In *Sensors* (Vol. 26, pp. 306).
- Wang, G., Sun, G., Wang, Q., Wang, S., Sun, H., & Xin, Q. 2010. Effect of carbon black additive in Pt black cathode catalyst layer on direct methanol fuel cell performance. *International Journal of Hydrogen Energy* 35(20): 11245–11253.
- Wang, G., Xu, X., Kou, X., Liu, X., Dong, X., Ma, H., & Wang, D. 2021. N-Doping of Graphene Aerogel as a Multifunctional Air Cathode for Microbial Fuel Cells. *ACS Applied Materials & Interfaces* 13(43): 51312–51320.
- Wang, J., Song, X., Wang, Y., Zhao, Z., Wang, B., & Yan, D. 2017. Effects of electrode material and substrate concentration on the bioenergy output and wastewater treatment in air-cathode microbial fuel cell integrating with constructed wetland. *Ecological Engineering* 99: 191–198.
- Wang, J., Yan, B., Zhang, H., Huang, L., Wang, H., Lan, Q., Yin, M., Zhu, Z., Yan, X., Zhu, A., Fu, C., & Wu, Y. 2022. Heavy Metals Exacerbate the Effect of Temperature on the Growth of *Chlorella* sp.: Implications on Algal Blooms and Management. In *Processes* (Vol. 10).
- Wang, N., Ma, S., Zuo, P., Duan, J., & Hou, B. 2021. Recent Progress of Electrochemical Production of Hydrogen Peroxide by Two-Electron Oxygen Reduction Reaction. *Advanced Science* 8(15): 2100076.
- Wang, P., Yang, F., Fan, H., & Lu, G. 2023. Bio-inspired multi-cell tubular structures approaching ideal energy absorption performance. *Materials & Design* 225: 111495.
- Wang, S., Adekunle, A., Tartakovsky, B., & Raghavan, V. 2021. Synthesizing developments in the usage of solid organic matter in microbial fuel cells: A review. *Chemical Engineering Journal Advances* 8: 100140.
- Wang, T., Chutia, A., Brett, D. J. L., Shearing, P. R., He, G., Chai, G., & Parkin, I. P. 2021. Palladium alloys used as electrocatalysts for the oxygen reduction reaction. *Energy & Environmental Science* 14(5): 2639–2669.
- Wang, Y., Li, K., Wang, Y., Liu, X., & Mu, Y. 2022. Nutrient limitation regulates the properties of extracellular electron transfer and hydraulic shear resistance of electroactive biofilm. *Environmental Research* 212: 113408.
- Wei, J., Meng, J., Zhang, C., Liu, Y., & Jiao, N. 2024. Dioxygen compatible electron donor-acceptor catalytic system and its enabled aerobic oxygenation. *Nature Communications* 15(1): 1886.
- Wu, D., Zhu, J., Zheng, Y., & Fu, L. 2024. Electrochemical Sensing Strategies for Synthetic Orange Dyes. In *Molecules* (Vol. 29, pp. 5026).
- Wu, J., Hu, K., Cheng, Y., Zhu, H., Shao, X., & Wang, Y. 2020. Data-driven remaining useful life prediction via multiple sensor signals and deep long short-term memory neural network. *ISA Transactions* 97: 241–250.

- Wu, J., Yan, G., Zhou, G., & Xu, T. 2014. Wastewater COD biodegradability fractionated by simple physical–chemical analysis. *Chemical Engineering Journal* 258: 450–459.
- Wu, X., Ren, X., Owens, G., Brunetti, G., Zhou, J., Yong, X., Wei, P., & Jia, H. 2018. A Facultative Electroactive Chromium(VI)-Reducing Bacterium Aerobically Isolated From a Biocathode Microbial Fuel Cell. *Frontiers in Microbiology* Volume 9 - 2018.
- Xie, X., Hu, J., Cao, X., Zhang, S., Sakamaki, T., & Li, X. 2024. In Situ Utilization of Electron-Enhanced Degradation of Azo Dyes in a Constructed Wetland–Microbial Fuel Cell Coupling System. In *Sustainability* (Vol. 16, pp. 3181).
- Yadav, S., & Patil, S. A. 2020. Microbial electroactive biofilms dominated by *Geoalkalibacter* spp. from a highly saline–alkaline environment. *npj Biofilms and Microbiomes* 6(1): 38.
- Yamamoto, M., Takaki, Y., Kashima, H., Tsuda, M., Tanizaki, A., Nakamura, R., & Takai, K. 2023. In situ electrosynthetic bacterial growth using electricity generated by a deep-sea hydrothermal vent. *The ISME Journal* 17(1): 12–20.
- Yamashita, T., Ookawa, N., Ishida, M., Kanamori, H., Sasaki, H., Katayose, Y., & Yokoyama, H. 2016. A novel open-type biosensor for the in-situ monitoring of biochemical oxygen demand in an aerobic environment. *Scientific Reports* 6(1): 38552.
- Yang, Q., Xu, L., He, Q., & Wu, D. 2022. Reduced cathodic scale and enhanced electrochemical precipitation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  by a novel fenced cathode structure: Formation of strong alkaline microenvironment and favorable crystallization. *Water Research* 209: 117893.
- Yang, Q., Yang, S., Liu, G., Zhou, B., Yu, X., Yin, Y., Yang, J., & Zhao, H. 2021. Boosting the anode performance of microbial fuel cells with a bacteria-derived biological iron oxide/carbon nanocomposite catalyst. *Chemosphere* 268: 128800.
- Yang, W., Chata, G., Zhang, Y., Peng, Y., Lu, J. E., Wang, N., Mercado, R., Li, J., & Chen, S. 2019. Graphene oxide-supported zinc cobalt oxides as effective cathode catalysts for microbial fuel cell: High catalytic activity and inhibition of biofilm formation. *Nano Energy* 57: 811–819.
- Yang, W., & Chen, S. 2020. Biomass-Derived Carbon for Electrode Fabrication in Microbial Fuel Cells: A Review. *Industrial & Engineering Chemistry Research* 59(14): 6391–6404.
- Yang, X., Zhou, Y., Zhang, L., Benally, C., & Liu, Y. 2025. Enhanced biofilm formation and municipal wastewater treatment efficiency using granular activated carbon modified bio-ball carriers in moving bed biofilm reactor. *Bioresource Technology* 435: 132947.
- Yang, Y., Xu, M., Guo, J., & Sun, G. 2012. Bacterial extracellular electron transfer in bioelectrochemical systems. *Process Biochemistry* 47(12): 1707–1714.
- Yang, Z.-C., Cheng, Y.-Y., Zhang, F., Li, B.-B., Mu, Y., Li, W.-W., & Yu, H.-Q. 2016. Rapid Detection and Enumeration of Exoelectrogenic Bacteria in Lake Sediments and a Wastewater Treatment Plant Using a Coupled WO<sub>3</sub>

- Nanoclusters and Most Probable Number Method. *Environmental Science & Technology Letters* 3(4): 133–137.
- Yeo, R. Y. Z., Ang, W. L., Mahmoudi, E., Ismail, M., Abu Bakar, M. H., Othman, A. R., & Lim, S. S. 2024. Enrichment of electrogenic microbes on surface-modified stainless steel 304L for rapid start-up of microbial electrochemical sensors. *Materials Today: Proceedings*.
- Yeo, R. Y. Z., Chin, B. H., Hil Me, M. F., Chia, J. F., Pham, H. T., Othman, A. R., Mohammad, A. W., Ang, W. L., & Lim, S. S. 2023. Rapid Surface Modification of Stainless Steel 304L Electrodes for Microbial Electrochemical Sensor Application. *ACS Biomaterials Science & Engineering* 9(11): 6034–6044.
- Yoo, H., Jo, H., & Oh, S. S. 2020. Detection and beyond: challenges and advances in aptamer-based biosensors. *Materials Advances* 1(8): 2663–2687.
- Yu, C., Wang, H., Yao, J., Zhao, J., Sun, Q., & Zhu, H. 2020. A dynamic alarm threshold setting method for photovoltaic array and its application. *Renewable Energy* 158: 13–22.
- Zajdel, T. J., Baruch, M., Méhes, G., Stavrinidou, E., Berggren, M., Maharbiz, M. M., Simon, D. T., & Ajo-Franklin, C. M. 2018. PEDOT:PSS-based Multilayer Bacterial-Composite Films for Bioelectronics. *Scientific Reports* 8(1): 15293.
- Zang, Y., Cao, B., Zhao, H., Xie, B., Ge, Y., Liu, H., & Yi, Y. 2023. Mechanism and applications of bidirectional extracellular electron transfer of *Shewanella*. *Environmental Science: Processes & Impacts* 25(12): 1863–1877.
- Zhang, B., Cheng, H.-Y., & Wang, A. 2021. Extracellular electron transfer through visible light induced excited-state outer membrane C-type cytochromes of *Geobacter sulfurreducens*. *Bioelectrochemistry* 138: 107683.
- Zhang, F., Xu, W., Zhang, L., Xi, L., Du, Y., Ma, L., Chen, S., & Du, D. 2022. Riboflavin as a non-quinone redox mediator for enhanced Cr(VI) removal by *Shewanella putrefaciens*. *Journal of Molecular Liquids* 351: 118622.
- Zhang, H., Lin, C., Sepunaru, L., Batchelor-McAuley, C., & Compton, R. G. 2017. Oxygen reduction in alkaline solution at glassy carbon surfaces and the role of adsorbed intermediates. *Journal of Electroanalytical Chemistry* 799: 53–60.
- Zhang, J., Zou, T., & Lai, Y. 2021. Novel method for industrial sewage outfall detection: Water pollution monitoring based on web crawler and remote sensing interpretation techniques. *Journal of Cleaner Production* 312: 127640.
- Zhang, P., Zhou, X., Qi, R., Gai, P., Liu, L., Lv, F., & Wang, S. 2019. Conductive Polymer–Exoelectrogen Hybrid Bioelectrode with Improved Biofilm Formation and Extracellular Electron Transport. *Advanced Electronic Materials* 5(8): 1900320.
- Zhang, Y., Gong, H., Liu, Q., Zhou, S., Zhu, D., & Dai, X. 2024. Stratified structure of membrane clogs observed in full scale cross-flow tubular ultrafiltration (UF) system: Fouling characterization and a proposed two-stage formation mechanism. *Water Research* 263: 122133.
- Zhang, Z., Yu, Y., Xi, H., & Zhou, Y. 2021. Single and joint inhibitory effect of nitrophenols on activated sludge. *Journal of Environmental Management* 294: 112945.

- Zhang, Z., Yu, Y., Xi, H., & Zhou, Y. 2022. Inhibitory effect of individual and mixtures of nitrophenols on anaerobic toxicity assay of anaerobic systems: Metabolism and evaluation modeling. *Journal of Environmental Management* 304: 114237.
- Zhao, J., Lin, J., Xiao, J., & Fan, H. 2015. Synthesis and electromagnetic, microwave absorbing properties of polyaniline/graphene oxide/Fe<sub>3</sub>O<sub>4</sub> nanocomposites. *RSC Advances* 5(25): 19345–19352.
- Zheng, S., Yang, F., Chen, S., Liu, L., Xiong, Q., Yu, T., Zhao, F., Schröder, U., & Hou, H. 2015. Binder-free carbon black/stainless steel mesh composite electrode for high-performance anode in microbial fuel cells. *Journal of Power Sources* 284: 252–257.
- Zhi, X., Vasileff, A., Zheng, Y., Jiao, Y., & Qiao, S.-Z. 2021. Role of oxygen-bound reaction intermediates in selective electrochemical CO<sub>2</sub> reduction. *Energy & Environmental Science* 14(7): 3912–3930.
- Zhong, J.-Q., Yan, K.-J., Yang, J., Yang, W.-H., & Yang, X.-D. 2022. Microenvironment Alters the Oxygen Reduction Activity of Metal/N/C Catalysts at the Triple-Phase Boundary. *ACS Catalysis* 12(15): 9003–9010.
- Zhou, E., Lekbach, Y., Gu, T., & Xu, D. 2022. Bioenergetics and extracellular electron transfer in microbial fuel cells and microbial corrosion. *Current Opinion in Electrochemistry* 31: 100830.
- Zhou, L., Liao, C., Li, T., An, J., Du, Q., Wan, L., Li, N., Pan, X., & Wang, X. 2018. Regeneration of activated carbon air-cathodes by half-wave rectified alternating fields in microbial fuel cells. *Applied Energy* 219: 199–206.
- Zhou, T., Han, H., Liu, P., Xiong, J., Tian, F., & Li, X. 2017. Microbial Fuels Cell-Based Biosensor for Toxicity Detection: A Review. In *Sensors* (Vol. 17).
- Zhu, K., Ren, X., Sun, X., Zhu, L., & Sun, Z. 2019. Effect of Supporting Electrolyte on the Surface Corrosion and Anodic Oxidation Performance of Graphite Electrode. *Electrocatalysis* 10(5): 549–559.
- Zhu, P., Zhuo, S., Zhang, W., Ying, X., Huang, J., & Li, X. 2023. Performance and application of microbial fuel cells with sodium alginate/agar/activated carbon composite as efficient biocompatible anode. *Journal of Applied Polymer Science* 140(26): e53999.
- Zhu, X., & Logan, B. E. 2014. Copper anode corrosion affects power generation in microbial fuel cells. *Journal of Chemical Technology & Biotechnology* 89(3): 471–474.
- Zhuang, S., Shao, C., Ye, J., Li, B., & Wang, X. 2020. Enhancing oxygen reduction reaction in air-cathode microbial fuel cells treating wastewater with cobalt and nitrogen co-doped ordered mesoporous carbon as cathode catalysts. *Environmental Research* 191: 110195.

## APPENDIX A

### LIST OF PUBLICATIONS

Parts of the data from this research thesis has been published in journals as indicated below:

Yeo, R. Y. Z., Ang, W. L., Bakar, M. H. A., Ismail, M., Salehmin, M. N. I., Yu, E. H., & Lim, S. S. 2025. Multi-Array Tubular Microbial Fuel Cell-Based Biosensor with Membrane-Electrode Assembled Air-Cathodes. *Fuel Cells* 25(1): e202400035.

Yeo, R. Y. Z., Ang, W. L., Wong, W. Y., Yu, E. H., Ng, Q. H., Goh, S. W., Salehmin, M. N. I., Mohamed, H., & Lim, S. S. 2025. A critical review on graphitic carbon nitride-based electrodes in microbial fuel cells for bioelectricity generation and bioremediation. *Journal of Environmental Chemical Engineering* 13(5): 117756.

Yeo, R. Y. Z., Chin, B. H., Hil Me, M. F., Chia, J. F., Pham, H. T., Othman, A. R., Mohammad, A. W., Ang, W. L., & Lim, S. S. 2023. Rapid Surface Modification of Stainless Steel 304L Electrodes for Microbial Electrochemical Sensor Application. *ACS Biomaterials Science & Engineering* 9(11): 6034-6044.