Doctoral Dissertation

Determination of Optimum Operating Condition for High Performance of Both Power Generation and Organic Removal in Dual-Chamber Microbial Fuel Cell

(二槽型微生物燃料電池における発電量と有機物除去双方の高効率化のための最適運転条件の検討)

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ABSTRACT

Population growth drives the demand for energy, which is the most pressing human need today. It puts pressure on other related sectors and increases wastewater production, which is a big problem in some countries that is inaccessible to available wastewater treatment facilities. Concerning the SDGs 2030 target, which mentions integrated sectors to deal with environmental issues, energy and water as a part of the water-energy-food nexus have a high linkage to build substantial economic and ecological benefits. Therefore, they have become the core attention of the world at this moment.

To deal with the problem solving mentioned above, nowadays, an alternative energy converter integrated wastewater treatment has been massively developed to achieve green label production of products. Many countries, favoured by their scientists and practitioners, compete to find technology that is a user-friendly, eco-friendly, back-to-nature concept, inexpensive and can be accepted in any society stratifications. However, there is an available standalone converter technology known as conventional technology, which is a disintegrated system with no added value. This challenge opens an opportunity to develop a fully integrated system with any advantages. One of the promising technologies needed for solving the environmental problem and simultaneously producing other benefits for human living is a part of the bioelectrochemistry system (BES), which is microbial fuel cell (MFC). It could be easily assisted with the available conventional wastewater treatment, and it gives more benefits not only in energy production but also for remediating the environment through the superior biocatalyst, named electroactive bacteria (EAB), which has the availability to reduce organic and inorganic matter and generating electricity. However, factors affecting MFC have been a drawback in their field application that must be concerned extensively. Therefore, this study accommodates to investigation more in order to get an optimum condition in operation so that the technology could be widely used properly on the full scale.

The disadvantages of the air–cathode single-chamber microbial fuel cell (AC-SCMFC) performance can be caused by numerous factors, and retention time (RT) is one such factor. It is difficult to conclude the ideal RT run for the specific tests under the same conditions. To determine the optimum RT for various types of microbial fuel cell (MFC), an AC-SCMFC batch-mode reactor was carried out by comparing different types and concentrations of substrates based on the main parameters of organic removal and power generation. The AC-SCMFC reactor was designed for the effective working volume of 500 mL and operated for 52 d in batch mode with factors being significantly correlated with the performance of the MFC reactor, which were two different substrates, sucrose and acetate, and three different chemical oxygen demand (COD) levels of 400; 1000, and 2500 mg/L (low, medium, and high, respectively) equipped with two graphene nanoplatelets (GNPs)-based electrodes connected to 100 Ω resistance and plugged onto a data logger. The results of this study indicated a significant pattern at the medium level, at which the optimum RT of sucrose was achieved at 24 h and that of acetate at 48 h. In comparison, the performances pattern at low and high levels of both substrates was insignificant to determine the optimum RT. For further application, the recommended RT for both substrates at any concentration is 24 h due to high overall performance, and the optimum RT established in this study could be applied to all types of MFC research, particularly in oxidizable or biodegradable organic ranges, which ensures high performance.

One of the important factors in enhancing the performance of microbial fuel cells (MFCs) is reactor design and configuration. Therefore, this study was conducted to evaluate the regressors and their operating parameters affecting the double anode chamber–designed dual-chamber microbial fuel cell (DAC-DCMFC) performance. Its primary design consists of two anode chamber compartments equipped with a separator and cathode chamber. The DAC-DCMFCs were parallelly operated over 8 days (60 days after the acclimation period). They were intermittently pump-fed with the different organic loading rates (OLRs), using chemically enriched sucrose as artificial wastewater. The applied OLRs were adjusted at low, medium, and high ranges from 0.4 $kg.m^{-3}.d^{-1}$ to 2.5 $kg.m^{-3}.d^{-1}$. The reactor types were type 1 and type 2 with different cathode materials. The pH, temperature, oxidation-reduction potential (ORP), optical density 600 (OD600), chemical oxygen demand (COD), and total organic carbon (TOC) were measured, using standard analytical instruments. In general, the power production achieved a maximum of 866 ± 44 mW/m^2, with a volumetric power density of 5.15 ± 0.26 W/m^3 and coulombic efficiency of 84%. Two-stage COD and TOC removal at medium OLR achieved a range of 60–80%. Medium OLR is the recommended level to enhance power production and organic removal in DAC-DCMFC. The separated anode chambers into two parts in a dual anode chamber microbial fuel cell adjusted by various organic loadings expressed a preferable comprehension of the integrated MFCs for wastewater treatment.

With respect to both studies, RT influences the design and configuration of MFCs, particularly in this regard, modified anode compartment of DCMFCs adapted to the range of oxidizable or biodegradable organics and reactor components towards control and dependent variables provide the simultaneous performance of DCMFCs in organic removal and power generation. In addition, DAC-DCMFC offers an opportunity to achieve optimal conditions in concurrent MFC-assisted wastewater treatment. Therefore, this study is one step closer to understanding the operating conditions comprehensively, which are the dominant factors affecting performance.

概要

現在、人口の増加がエネルギー需要を押し上げているが、各種エネルギーは今日において、人類の存続のために必要不可欠なものである。現状において採択されているSDGs 2030の目標とのその関連で、ある部門が他の関連部門の負担を増加させ、廃水発生量を増加させるため、利用可能な廃水処理施設に十分にアクセスできない国もあり大きな問題となっている。この環境問題に対処するために総合的な視点から俯瞰すると、水・エネルギー・食糧問題と強い因果関係のあるエネルギーと水環境の改善は、実質的に経済的・生態学的利益を構築するための高い連動性を持っており、今日において世界的に大きな注目を集めている。

前述のような問題の解決に対応するため、昨今、グリーン（環境）ラベル製品の生産実現への努力と代替エネルギーコンバーター一体型廃水処理装置の開発が盛んに行われており、これは多くの国々において、科学者や実業家の支持を受け、ユーザーや環境に配慮した自然に回帰するというコンセプトの下で、安価にすべての人々に提供可能な技術を求めて競争が行われている。

単体コンバーター技術は従来技術として知られているが、これは他に付加価値を持たない細分化されたシステムである。本研究の目標は、あらゆる利点を備えた完全統合型システムを開発する機会を開くことに貢献することである。環境問題を解決し、同時に人の生活にも役立つ技術として期待されているのが、バイオエレクトロケミストリーシステム（BES）の一部である微生物燃料電池（MFC）である。このMFCは電気活性（生産）細菌（EAB）という有機物や無機物を還元して発電することができる優れた生体触媒により、従来の廃水処理に容易に対応でき、エネルギー生産だけでなく環境修復の面でも大きなメリットを有する。しかし、MFCを実用化（実プラントに適用）する際には、まずはMFCに影響を与える様々な要因を明らかにする必要があり、広く条件を検討する必要がある。そこで、本研究ではMFCを本格的に普及させるために、最適な運転条件を得るための検討を行った。

空気陰極（エアカソード）単室型微生物燃料電池（AC-SCMFC）の性能的欠点は、複数の要因によって生じる可能性があり、保持時間（RT）はその要因のうちの1つである。同じ条件下で特定の試験によって得られた結果から理想的な滞留時間を結論づけることは一般に困難であるため、様々なタイプの微生物燃料電池（MFC）に対して最適なRTを決定するために、有機物除去と発電の主要なパラメータに基づいて、基質の

種類と濃度の異なる AC-SCMFC リアクター（バッチモード）の運転結果を比較することにより実施した。AC-SCMFC リアクターは有効容量 500 mL で設計されており、本研究ではバッチモードで 52 日間運転を行った。実験条件は、本リアクター（100 Ω 抵抗に接続されデータロガーに接続された 2 つのグラフェンナノプレート（GNP）ベース電極を備える）を用いて、スクロースと酢酸の 2 種類の基質についてそれぞれ 400、1000、2500 mg/L （それぞれ低濃度、中濃度、高濃度）という 3 種類の COD（化学的酸素要求量）レベルを設定した。実験結果から、中濃度の COD レベルでは，スクロース基質の場合の最適な RT は 24 時間，酢酸の最適な RT は 48 時間にであった。一方で、低濃度および高濃度 COD レベルでの実験結果からは、最適な RT を決定することが困難であった。これらの結果から、いずれの基質濃度においても RT を 24 時間とすることで総合的な性能向上が見込まれることが示された。本実験で確立した最適な RT は、あらゆるタイプの MFC、特に酸化性または生分解性の有機領域で適用でき、高い性能を確保できることが期待される。

微生物燃料電池（MFC）の性能を向上させる重要な要素の一つに、リアクターの設計と構成がある。そこで、本研究では前述の AC-SCMFC に加えてダブルアノードチャンバーを備えたデュアルチャンバー型微生物燃料電池（DAC-DCMFC）の性能に影響を与える要因とその影響を把握・評価するための実験を実施した。DAC-DCMFC の主要な構造は、セパレータとカソードチャンバーを備えた 2 つのアノードチャンバーコンパートメントで構成されている。DAC-DCMFC は 8 日間（馴化期間から 60 日後に 8 日間）にわたって並行して運転を行った。運転に際して、人工廃水として基質としてスクロースを用い、異なる有機物負荷（OLR）で間欠的にポンプにより送液した。適用した OLR は 0.4 $kg.m^{-3}.d^{-1}$ から 2.5 $kg.m^{-3}.d^{-1}$ まで低、中、高の 3 パターンで調整し、さらに、正極材が異なるタイプ 1 とタイプ 2 を用いた。pH，温度，酸化還元電位（ORP）， OD600，化学的酸素要求量（COD：クロム法）， 全有機炭素（TOC）について測定した。実験結果から、発電量は最大 866±44mW/m^2、体積出力密度 5.15±0.26W/m^3、クーロン効率 84%が得られた。同時に、この中パターンの OLR における COD および TOC 除去率は、60～80%の範囲を達成し、このパターンの OLR は、DAC-DCMFC の発電量と有機物除去率を両立させることのできる範囲であることが示唆された。これにより、様々な有機物負荷に対応できる DAC-DCMFC は、アノードチャンバーを 2 つに分離することで、第 1 段で高濃度に対応し、第 2 段で中低濃度に対応することができ、良好な発電量も得られることが示された。これらの情報は MFC 研究に対する知見の集積という面からも有益であると考えられる。

以上の 2 つの実験によって、①得られた最適な RT は、あらゆるタイプの MFC、特に酸化性または生分解性の有機領域で適用でき、高い性能（排水処理効率と発電効率）を確保できることが期待でき、②様々な有機物負荷に対応できる DAC-DCMFC は、アノードチャンバーを 2 つに分離することで、第 1 段で高濃度に対応し、第 2 段で中低濃度に対応することができ、さらに良好な発電量も得られることが示された。ることが示された。

以上から本研究の有用性が示されたと考えられる。

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LIST OF ABBREVIATIONS

BES	Bioelectrochemical system
BEAMR	Bioelectrochemical assisted microbial reactor; bioelectrochemically-assisted microbial reactor
AD	Anaerobic digestion
UASB	Up-flow anaerobic sludge blanket
ABR	Anaerobic baffled reactor
WWT	Wastewater treatment
WWTP	Wastewater treatment plant
LNG	Liquefied natural gas
MD	Membrane distillation
IEA	International energy agency
MDGs	Millennium development goals
SDGs	Sustainable development goals
WEF	Water-energy-food
OECD	Organisation for economic co-operation and development
FAO	Food and agriculture organization
EFC	Enzymatic fuel cell
MEC	Microbial electrochemical cell
MDC	Microbial desalination cell
MSC	Microbial solar cell
MFC-WWT	Microbial fuel cell-assisted wastewater treatment
MFC	Microbial fuel cell
SCMFC	Single chamber microbial fuel cell
DCMFC	Dual-chamber microbial fuel cell; double chamber microbial fuel cell
AC-SCMFC	Air cathode-single chamber microbial fuel cell

DAC-DCMFC	Double anode chamber designed dual-chamber microbial fuel cell
PMFC	Photosynthetic microbial fuel cells
FPMFC	Flat plate microbial fuel cell
UMFC	Upflow microbial fuel cell
MFC-CW	Microbial fuel cell-constructed wetland
RT	Retention time
HRT	Hydraulic retention time
SRT	Solids retention time
GNPs	Graphene nanoplatelets
GNPs:PU	Graphene nanoplatelets-water-based polyurethane
GNPs-PTFE	Graphene nanoplatelets-polytetrafluoroethylene
OLR	Organic loading rate
DO	Dissolved oxygen (mg O_2/L)
COD	Chemical oxygen demand (mg/L or mg COD/L)
TOC	Total organic carbon (mg/L)
pH	Potential of hydrogen
OD_{600}	Optical density with the wavelength 600nm (Abs)
ORP	Oxidation-reduction potential (mV)
BOD_5	Biological oxygen demand (mg/L)
P-PO_4	Orthophosphate phosphorus (mg/L)
P	Phosphorus
N-NO_3	Nitrate nitrogen (mg/L)
TS	Total solids (mg/L)
TSS	Total suspended solids (mg/L)
VSS	Volatile suspended solids (mg/L)
FSS	Fixed suspended solids (mg/L)
CO_2	Carbon dioxide

CH_4	Methane
H^+	Hydrogen ion
e^-	Electron
ORR	Oxygen reduction reaction
PP	Polypropylene
SSM	Stainless steel mesh
PU	Polyurethane (synthesized)
PTFE	Polytetrafluoroethylene
PEM	Proton exchange membrane
EAB	Electrochemically active bacteria; electroactive bacteria
EEB	Exoeletrogenic bacteria
EB	Electrogenic bacteria
EMC	Electrotrophic microbial consortia
EU-STP	Eastern-ube sewage treatment plant
BLASTN	Basic local alignment search tool nucleotide
NCBI	National center for biotechnology information
PCR	Polymerase chain reaction
DGGE	Denaturing gradient gel electrophoresis
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
DNA	Deoxyribonucleic acid
NPP	Nitrogenphosphate-potassium
Q	Flow rate
Vol.	Reactor volume
LWH	Length-width-height
So	Influent concentration (mg/L)
C	Concentration (mg/L)

Co	Initial concentration (mg/L)
t	Detention time (d)
k	First-order rate constant (/d)
d	Day(s)
h	Hour(s)
r	Removal (%)
p.a.	Pro-analysis
e	The base of the natural logarithms
I	Current; the electrical current intensity (A or mA)
V	Cell voltage (V or mV)
R	Resistance (Ω)
P	Power (W or Watt)
PD	Power density (W/m^2 or mW/m^2; W/m^3 or mW/m^3)
CD	Current density (A/m^2 or mA/m^2)
A	The surface area of the anode carbon (m^2)
CE	Coulombic efficiency (%)
F	Faraday’s constant (96485 C/mol electrons)
n	The actual electrons transferred (4 mmol of electron per mmol of COD)
Cs	The charge passed (A.s)
ΔE	Potential energy (mV)
M	The molecular weight of oxygen (g/mol)
z	The number of electrons exchanged per mole of oxygen
ΔCOD	The difference in the influent and effluent COD (g/L)
rCOD	The removal rate of COD (g/L)
F/M ratio	Food to microorganism ratio
v	The anodic volume (L)

Vanodic	The volume of anodic (L)
A	Anode surface area (m^2)
WV	Working volume (m^3)
X	Low OLR
Y	Medium OLR
Z	High OLR
1A1	Type 1 reactor and first anode chamber
2A1	Type 2 reactor and first anode chamber
1A2	Type 1 reactor and second anode chamber
2A2	Type 2 reactor and second anode chamber
S1	Low strength level of sucrose
S2	Medium strength level of sucrose
S3	High strength level of sucrose
A1	Low strength level of acetate
A2	Medium strength level of acetate
A3	High strength level of acetate
kJ	Kilojoule
OCV	Open-circuit voltage
CCV	Closed-circuit voltage
OM	Operational maintenance
J-NES	Japan national effluent standard
C/B	Cost-benefit ratio
Ɵc	Sludge age
Ω	Ohm
in	Input
out	Output
600	Wavelength value (nm)

t	Final
o	Initial or influent
int	Internal

CHAPTER I
INTRODUCTION

1.1. Background

Energy demand increases with population growth, suppressing an increase in other related sectors, such as wastewater generation, which has become a significant problem in developing countries. An amount of energy is largely consumed by conventional wastewater treatment plants (Capodaglio and Olsson, 2020; Ranieri, Giuliano and Ranieri, 2021). In addition, energy scarcity has become a worldwide concern because it is a big problem that must be solved appropriately. Altintas *et al.* (2020) noticed that the increasing need for energy and its related services to satisfy economic and social development has become a critical concern of national governments worldwide. Currently, un-renewable energy, from gasoline, diesel fuel, and coal, is still used for supporting anthropogenic activities. Since it was exploited improperly, it has occurred, particularly the use of nonrenewable energy sources, which contribute to almost 70% of global energy demand, with natural gas covering nearly 45% of total demand growth (IEA, 2021). Many countries, through their scientists, compete to find a proper alternative, particularly following back to nature concept, to answer the demand for energy. The main idea is to utilize renewable energy sources, which consist of sunlight, wind, biomass, etc. Currently, their use is increasing to complement the need for total energy. In this study, biomass plays an important role in favouring energy production through the help of energy-generating microorganisms, even though their energy production isn't high compared to other renewable energy sources. Bekun (2022) argues that it is a more environmentally friendly option for reaching environmental sustainability targets, particularly with regards to energy investments in collaboration with private partners. Interestingly, a part of biomass, wastewater containing sufficient carbons, nutrients, and trace elements, begins to be famously used in every biological process to yield energy, called bioenergy, and simultaneously remove wastewater pollutants. However, the utilization of wastewater still lacks field practice due to low targets in energy production, even though reaching a maximum target in wastewater pollutant removal complies with the national and international standards of wastewater discharge. Therefore, a new challenge appears to accomplish society's questions in energy fulfillment, and concurrently, solving the wastewater pollutant removal to create the integrated waste management and support the integrated SDGs 2030 program, which is number 7 about affordable and clean energy, number 6 about clean water and sanitation, number 13 about climate action, number 3 about good health and well being, and number 11 about sustainable cities and communities.

In order to acknowledge the challenge, this study is focused on developing microbial fuel cells (MFCs) both in single-chamber and dual-chamber types. MFC is a part of the bioelectrochemical system (BES), which has the ability to convert organic matter, such as single and complex substrates, into direct electricity without any additional generators and pure water. MFC can be integrated with the current biological wastewater treatment, named MFC-integrated wastewater, which treats low-strength wastewater downstream. Rahimnejad, Asghary and Fallah (2020) mentioned that MFC had become an innovative technology for wastewater treatment and power generation. However, some drawbacks still exist, which means some factors affect the performance of MFC, whilst this study effort to minimize their obstacle by focusing on modifying the design and configuration of both SCMFC and DCMFC types. Single-chamber MFC (SCMFC), reconstructed as an air-cathode SCMFC (AC-SCMFC), is employed to determine the retention time (RT) used to optimize the performance of any MFC studies, which is both in organic removal and power production. Meanwhile, dual-chamber MFC (DCMFC), redesigned as dual anode chamber DCMFC (DAC-DCMFC), is used to achieve high performance in MFC studies by implementing the optimum RT delivered from the study of RT in SCMFC. A substantial consideration of modifying both MFC types is easier to build and operate than other types of MFC. Therefore, both modifications of MFCs were carried out in parallel to obtain high performance in organic removal and power production by reducing their resistance and simultaneously providing recommendations for further studies.

RT plays an essential role in the process and hydrodynamic factors that affect wastewater treatment design and operation, respectively. Organic loading rate (OLR) and flow rate (Q), which are the principle of hydrodynamic factors to obtain reactor volume, is highly correlated to RT (Nawaz *et al.*, 2020). Regarding the process in MFC as the alternative to wastewater treatment, RT provides linear results with organic and inorganic removals, even if they are inverse to the current density for power generation (Malekmohammadi and Mirbagheri, 2021). Samudro, Imai, and Hung (2021) concerned that RT is the most highly influenced factor in MFC due to the relationship with the loading rate of substrates and reactor volume, which are expressed in fed-batch and continuous processes. There are many terms for RT, which are detention time (t), hydraulic retention time (HRT), solids retention time (SRT), and sludge age (Θ_c). t and HRT can be used to enumerate the reactor volume (V) for reactor design and the organic loading rate (OLR) for hydrodynamic operation. Whereas SRT and Θ_c are employed to calculate the sludge chamber volume of the biological wastewater treatment unit and discharge the amount of sludge after the treatment period. In this study, the primary study is to determine RT for designing and operating reactors in optimal conditions. RT is studied comprehensively in chapter III to obtain optimum conditions by varying the concentration and type of substrate to support the DAC-DCMFC design, which is discussed in chapter IV, where RT is locked as the dependent variable; while the type of reactor and sucrose-based substrate OLRs are set as independent variables. In order to obtain the optimum condition; therefore, the substrate factors are appointed due to highly correlated factors in many MFC studies. Whereas the reactor types are chosen due to the different effects in variant configurations.

DAC-DCMFC, a novel design and configuration of DCMFC variances, is performed to implement the optimum RT and recommended organic strength level resulting from operating AC-SCMFC. In addition, its purpose is to examine the influence of varying OLRs and reactor configuration types on several parameters, which mostly utilize indicators in MFC studies. According to its purpose, the recommended reactor type could be selected based on its high performance both in organic removal and power production. In order to achieve a more significant performance of power generation and organic removal, multiple anode chamber studies are employed extensively (Samudro, Imai, and Hung, 2021). There were many studies previously, such as those studied by Kim *et al.* (2011), about the performance of an anaerobic dual anode-chambered MFC, and Samsudeen *et al.* (2015), concerning the performance of multi-chamber MFC for the scale-up system. DAC-DCMFC is built to minimize the internal resistance causing several problems in the performance, particularly in power generation. The internal resistance of the conventional DCMFC is that the whole process takes place in the anode chamber so that several parameters, EAB species, anode material, separator, and configuration, can interfere with electricity production. Therefore, based on the idea mentioned by Aelterman *et al.* (2006), the fundamental of DCMFC is modifying an anode chamber into multiple chambers, known as stack MFC, or alternatively, DCMFC is employed as a downstream treatment for low strength levels of organic after treating high strength levels of organic through available anaerobically biological treatment such as anaerobic digestion (AD), up-flow anaerobic sludge blanket (UASB), anaerobic baffled reactor (ABR) without maturation, etc. In order to take into account the realization of the idea; subsequently, the anode chamber in DCMFC is separated into two chambers with the purpose of minimizing the internal resistance caused by substrate type and concentration, the degradation of organic substrates into solids, anode material, biofilm covered anode, microorganism species containing substrate, separator, and the distance between anode and cathode.

This study contributes to MFC studies in accordance with design criteria, RT of factors affecting MFCs, and alternative design of DCMFC variance, dual anode chamber designed DCMFC (DAC-DCMFC) in enhancing both in organic removal and power generation. In order to achieve the performance of MFC both in organic removal and power generation, this study was conducted to determine the optimum RT through an AC-SCMFC batch-mode reactor by comparing different types and concentrations of substrates based on the main parameters of organic removal and power generation, and evaluate the regressors and their operating parameters affecting the double anode chamber–designed dual-chamber microbial fuel cell (DAC-DCMFC) performance. The determined optimum RT established from AC-SCMFC can be used to design an anode chamber, either single or multiple anode chambers MFC, and concurrently evaluate the process of MFC performance pertaining to organic removal and power generation. Furthermore, The DAC-DCMFC is an implementation of the optimum RT function found in the AC-SCMFC study and is used for its design and operation. Organic removal parameters consist of COD removal (CODr), the reaction rate of COD (kCOD), TOC removal (TOCr), and the reaction rate of TOC (kTOC), whereas power generation parameters consist of cell voltage, PD, CE, and internal resistance. Factors affecting AC-SCMFC and DAC-DCMFC are assigned for the eight and five parameters mentioned above. In addition, TOC is employed as an advantage to ensure the COD result analysis and can be used to investigate the phenomenon of change or removal of organic matter contained in wastewater that occurs in both reactors.

1.2. Dissertation objectives

This study aims to simultaneously discover high-performance factors affecting MFCs in organic removal and power generation through AC-SCMFC and DAC-DCMFC. Five sub-objectives have been formulated to obtain the goal mentioned above:

1. To determine the optimum RT through an AC-SCMFC batch-mode reactor by comparing different types and concentrations of substrates based on the main parameters of organic removal and power generation.
2. To investigate the regressors and their operating parameters affecting the double anode chamber–designed dual-chamber microbial fuel cell (DAC-DCMFC) performance.
3. To recommend the optimal substrate types and concentration through performing AC-SCMFC for optimizing the performance of MFCs.
4. To suggest the optimal OLR of sucrose-based synthetic wastewater and reactor type through performing DAC-DCMFC.
5. To elaborate more on the policy recommendations to policymakers for the investigated bloc of both AC-SCMFC and DAC-DCMFC studies.

1.3. Structure of the dissertation

This dissertation is divided into five chapters and listed as follows:

- Chapter I states the rationale and background, objectives, and structure of the dissertation.
- Chapter II explores the extensive background of the theories, methods, and summary of previous studies.

- Chapter III determines the optimum RT through an AC-SCMFC batch-mode reactor by comparing different types and concentrations of substrates based on the main parameters of organic removal and power generation.
- Chapter IV evaluates the regressors and their operating parameters affecting the double anode chamber–designed dual-chamber microbial fuel cell (DAC-DCMFC) performance.
- Chapter V recapitulates the study results and offers suggestions for future studies.

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CHAPTER II
LITERATURE REVIEWS

2.1. Supply and Demand

2.1.1. Energy

Nowadays, the energy demand has become unbalanced compared to the energy supply. Gan et al. (2020) reported that world energy consumption has indicated large differences and imbalances and has a substantial correlation between the growth of socio-economic and the consumption of electricity per capita. Energy supply mostly comes from non-renewable energy, such as oil, coal, natural gas, etc. Khan et al. (2021) conclude that non-renewable energy takes a large proportion in utilization in developing countries to promote their economic growth. On the other hand, their capacity is limited due to high requests in some countries. Around 70% of energy supply comes from non-renewable sources, and 30% of them are from renewable sources. IEA (2021) noticed that the primary energy demand originates from oil, coal, gas, nuclear, and renewables, in which China has higher demand than India, the United States, the European Union, and the total world, as depicted in **Figure 2.1**. The third biggest demand is oil, coal, and renewables. Only the European Union has the biggest demand for nuclear besides renewables. Interestingly, they report that renewable energy has been used as the main energy source, which means it occupies the same proportion as non-renewable energy needs.

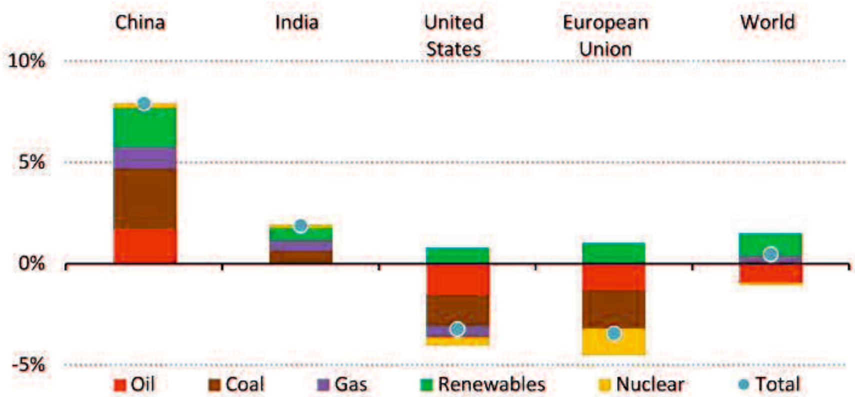

Figure 2.1 Change of primary energy demand by region and by fuel in 2021 relative to 2019. Credited to IEA (2021).

Many countries, especially developing countries, compete for renewable energy resources from many locations, but there are several constraints on developing them on a practical level. As reported, the use of renewable energy climbed up to 3% in 2020 as demand in which other energy sources declined (IEA 2021). Several renewable energy sources are described in **Figure 2.2**. Biomass, one of the renewables, is an abundant energy source provided by nature (Cuong et al. 2021; Zhao et al. 2021) and is rich in carbon and nutrients, which can be used for direct energy generation, although they produce lower energy than other renewable energy sources (Wu et al. 2018; Leger et al. 2021). Most countries, particularly developing countries, use less than 10% of total biomass energy sources annually (IEA 2021). Findings show that they are more environmentally friendly in terms of reaching environmental sustainability targets, particularly concerning energy-development investments in collaboration with private partners (Bekun 2022). The basic framework for balancing supply and demand in energy, particularly in renewable energy, is depicted in **Figure 2.3**. Hence, the development of renewable energy converters into several products, such as electricity, heat, etc., is emerging in order to fulfil the energy demand worldwide.

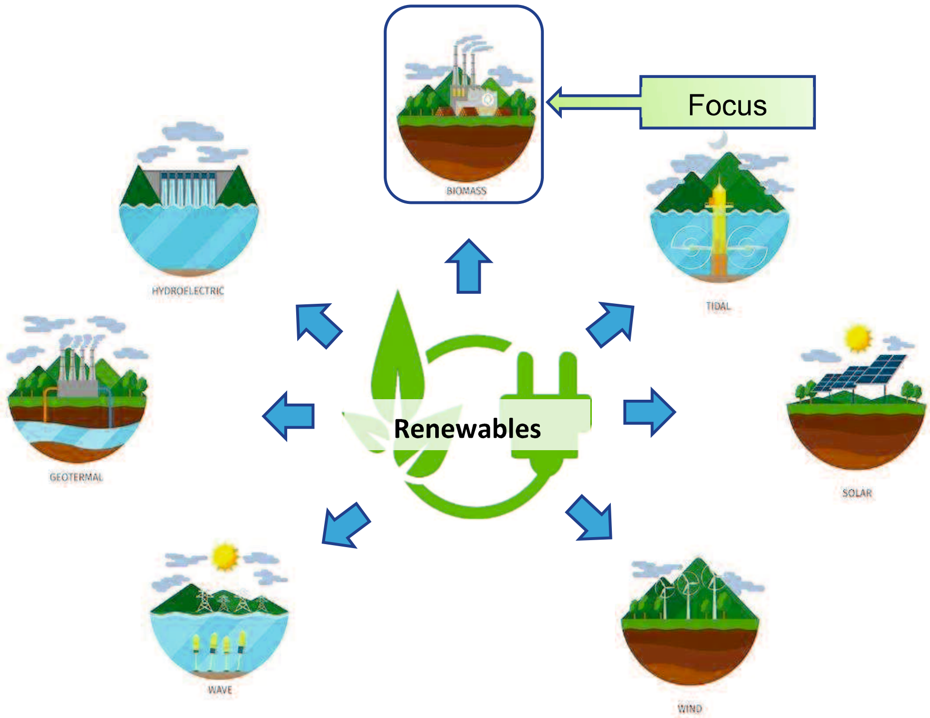

Figure 2.2 Renewables energy. Adopted elsewhere.

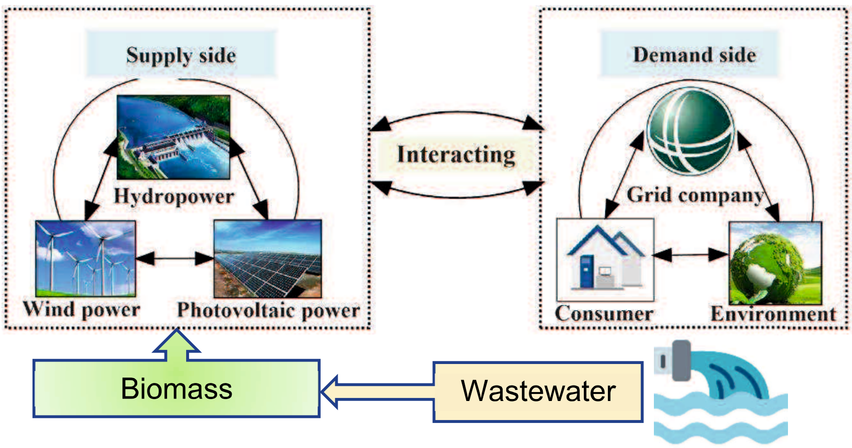

Figure 2.3 Basic framework for balancing supply and demand in energy. Adopted from Gan et al. (2020).

2.1.2. Water

Like energy demand, water plays a key important role in humankind. Water is used for any purpose for human activities or anthropogenic. Likewise, Jilito et al. (2020) mentioned that water plays a critical role to improve food security and livelihoods of rural communities. Therefore, water availability, both in quality and quantity, especially clean water and drinking water, must be ready at any time. Bain, Johnston, and Slaymaker (2020) mentioned that the sustainable development goals (SDGs) include ambitious global targets for drinking water, sanitation, and hygiene. Currently, we found that the two aspects are gradually declining year by year due to climate change and human behaviour. The cause is occurred mostly happens due to human activities. Like climate change, not only it happen naturally, but also human intervention impacts environmental degradation.

Water pollution is mainly caused by some accidental substances entering the water and impacting the quality. In addition, Qadri et al. (2020) noticed that water pollution is a pressing environmental concern due to ever-increasing anthropogenic pressures. The unexpected substances could be originated from organic, heavy metals, salt, radioactive, and microbiology matters. Surprisingly, anthropogenic substances, such as pharmaceuticals or antibiotics and some related ones, of substantial concern affecting aquatic ecosystems, appear in wastewater (Moslah et al. 2018; Ikonen et al. 2021). However, the primary cause is their concentrations exceeding the national or international standard. Therefore, the polluted water should be recovered by using appropriate technology.

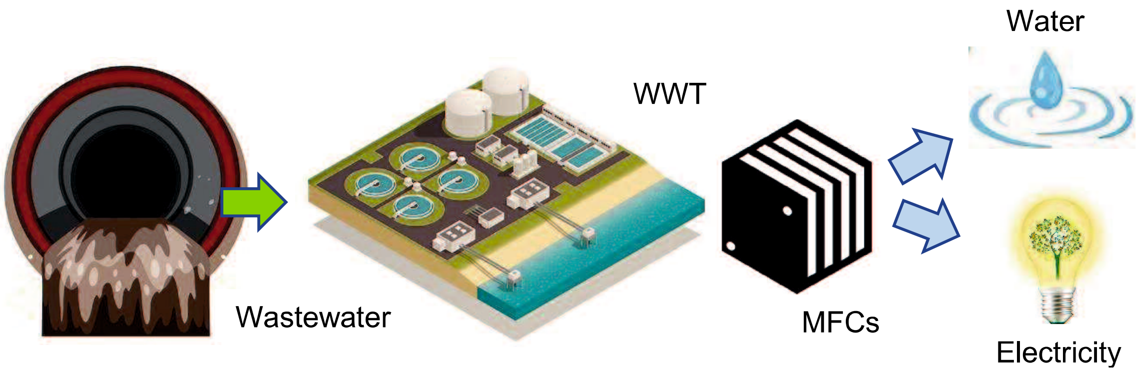

Figure 2.4 Wastewater converted into renewable energy

In accordance with the waste-to-energy concept; thus, water pollution could be used as a source of essential matter, like carbon, nutrients, and other trace elements, as described in **Figure 2.4**. This study promotes the use of organic matter containing water pollution to be utilized as one of the unexpected biomass abundantly in many countries correlated with high population density. Duijn et al. (2019) noticed that community-based initiatives are emerging in many domains, such as care, sustainable energy and water management. Therefore, water pollution as wastewater is used to generate electricity as green energy and, at the same time, minimize unexpected substances through the basic concept of wastewater treatment.

2.2. Water-Energy-Food (WEF) Security Approach

Population plays a vital role in anthropogenic sectors, such as energy, food, water, etc. Water-energy-food becomes a security nexus concept pertaining to one another that can able to affect one or both of the other areas. A substantial relation among them creates highly correlated sectors in which water, energy, and food are utilized and needed for extraction, processing, transporting, and distributing, respectively, and employed for treating water and growing plants for food. Nexus security principles related to the water-energy-food security approach are depicted in **Figure 2.5**. In addition, water and energy security are defined in the Millennium Development Goals (MDGs), and currently, it is mentioned in the Sustainable Development Goals (SDGs) (de Jong and Vijge 2021); meanwhile, food security is established by the Food and Agriculture Organization (FAO) (OECD/FAO 2016). As they become an important factor in their functioning and worldwide agreement, an appropriate method approach to implementation should be required to support their performance in many countries.

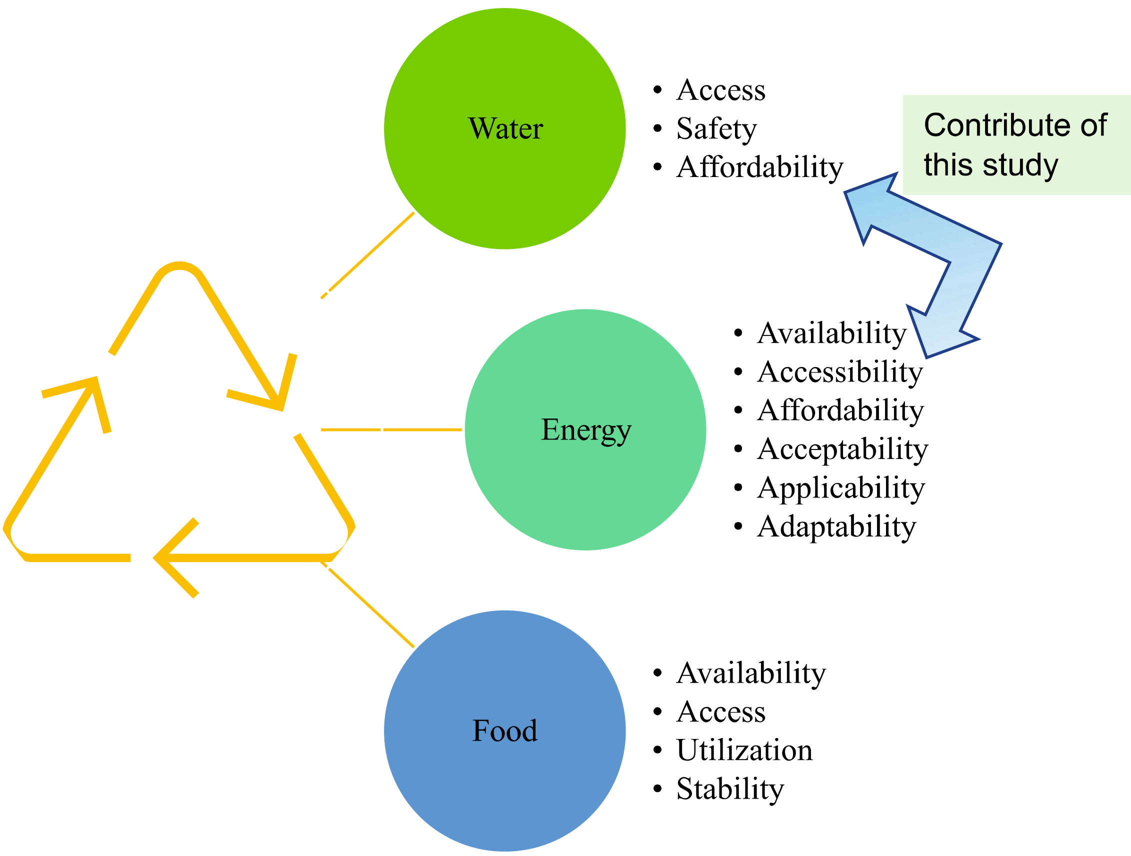

Figure 2.5 Nexus security principles. Adopted from Fetanat et al. (2021).

Specifically, water and energy as the focus of this study, energy and water have grown to be the centre of the world's attention, particularly for sustainable development. Energy and water support human and living things' activities to produce their demand (H. Samudro and Mangkoedihardjo 2021; G. Samudro and Mangkoedihardjo 2020; Syvitski et al. 2020). Hence, energy and water cannot be separated due to their synergism creating significant economic and ecological benefits. Regarding the benefits of water utilization in society, the integrated energy source with water is described in **Figure 2.6**.

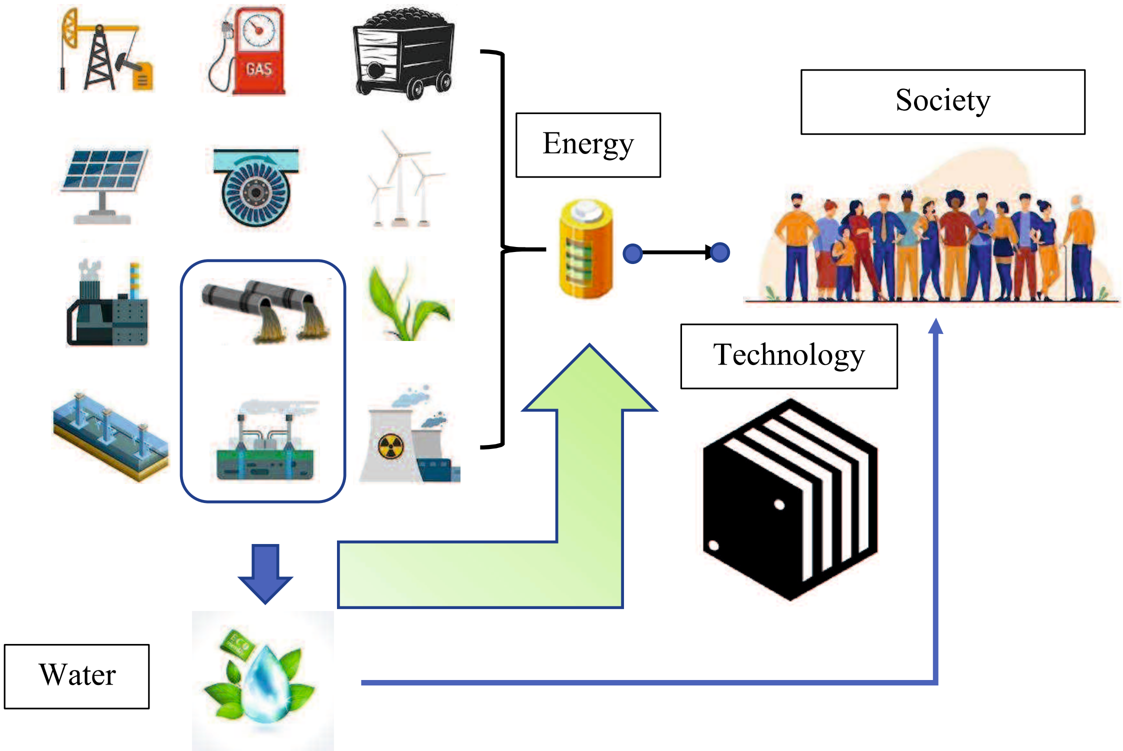

Figure 2.6 Integrated energy source with water utilization that benefits the community

Nowadays, the nexus approach has been applied to several methods of environmental recovery technology. For water recovery, the optional wastewater treatment technology can be nominated by using a comprehensive decision-making framework under an intuitionistic fuzzy environment (Fetanat, Tayebi, and Mofid 2021). In addition, other methods have been proposed in many references, i.e., innovation by coupling LNG cold energy with HyDesal in order to strengthen the water-energy nexus (Babu et al. 2018), desalinating high-salinity waters using low-grade or waste heat in membrane distillation (MD) (Deshmukh et al. 2018), two-stage microbial degradation process in which both the production of keratinolytic enzymes and hydrolysis of keratinous waste (Udugama et al. 2020), etc.

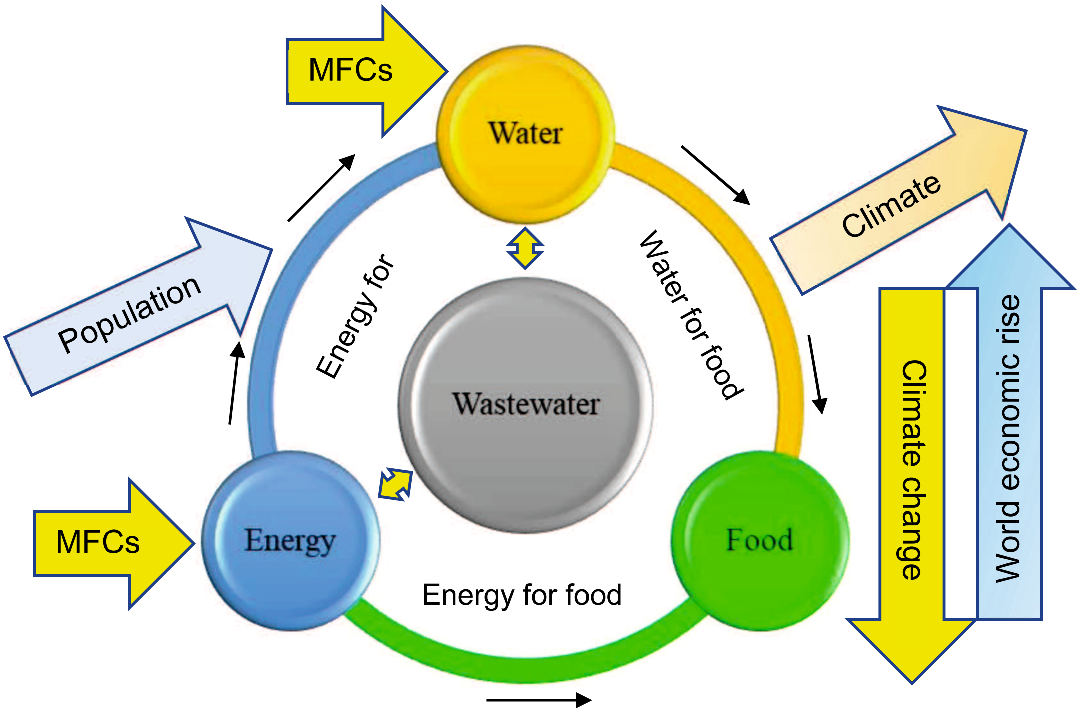

Figure 2.7 The concept of MFC's contribution to nexus implementation. Adopted from elsewhere.

This study implements microbial fuel cells (MFCs) that support the nexus of the water-energy security approach as described in **Figure 2.7**; however, some limitations open the opportunity to be developed in the future. The main boundary of MFCs is their sustainable application on a large scale due to a well-known new method in the integrated wastewater for recovery and power production. Abdullah et al. (2019) criticize that MFC technology is still largely stuck in laboratory experiments studying its performance and potential for improvement. On the other hand, MFCs for power generation cannot be utopian as long as they are integrated into the structure of a central wastewater treatment plant (Tsekouras et al. 2022). However, the development of MFCs attracts many scientists worldwide to compete in finding the appropriate method, including electrode materials that are the most influential factor besides electroactive bacteria (EAB), for the full-scale purpose. Hence, MFCs can be classified as the most considered technology in supporting the implementation of the WEF nexus, particularly the water-energy security nexus.

2.3. Wastewater Treatment

Wastewater means polluted water containing high organic and inorganic substances that exceed national and or international standards. Wastewater can be divided into two-general types, which domestic and industrial wastewater. Domestic wastewater mainly originates from public or private houses and small-scale trade houses, whereas industrial wastewater primarily comes from mid to large-scale houses and industry regions. Regarding the wastewater characteristics, both domestic and industrial wastewater contributes to the extended range concentration of organic substances; likewise, they augment a part of inorganic substances even in short-range concentration that is mainly classified as toxic matter. Organic substrate concentrations are primarily defined as low (250–750 mgCOD/L), medium (>750–2250 mgCOD/L), and high (>2250 mg COD/L) strength levels (COD: chemical oxygen demand) (Stoll, Dolfing, and Xu 2018). They further explained that a COD concentration of more than 2,250 mg/l is categorized as high-strength wastewater, including industrial waste such as brewery and food processing wastewater. Whereas COD concentration lower than 2,250 mg/l is classified as medium and low-strength wastewater, including various domestic wastewater. Hence, their concentration range was used as a benchmark for this study in order to contribute to similar conditions. Meanwhile, the inorganic substrate concentrations are defined as an experiment limitation since microbial utilization is highly influenced by inorganic substances, heavy metals and salt, even in a small amount of concentrations.

Wastewater treatment aims to remove or even minimize a part of substance contents using natural and engineered methods to deal with the national and or international effluent standards before discharging to the environment. Furthermore, wastewater treatment primarily reduces organic and inorganic substances producing by-products that contribute to a multi-dimensional environment; however, some drawbacks occur when the by-products are only moved from the initial medium to other mediums without any other values. Hence, advanced wastewater treatment has been recently developed to meet the requirements. For example, modifying wastewater treatments with an energy converter in which they are not only removing organic and inorganic substances but also producing bioenergy. They are well-known as Anaerobic Digestion (AD), Up-flow Anaerobic Sludge Blanket (UASB) and An-aerobic Baffled Reactor (ABR), which are able to generate biogas (Fetanat, Tayebi, and Mofid 2021), and Microbial Fuel Cell (MFC), which is able to produce electricity directly (Fetanat, Tayebi, and Mofid 2021), and a small amount of biogas and water. Therefore, MFC is focused on this study for the reasons mentioned above. Even though MFC was not able to generate a large amount of biogas, it can be overcome to generate electricity directly without additional converters and simultaneously reduce the high-medium strength level of organic matter contained in the wastewater.

2.4. Microbial Fuel Cells (MFCs)

2.4.1. General

MFCs are a part of bioelectrochemical systems (BESs), which can convert chemical energy from organic matter, ranging from complex to simple biomass, into electrical energy, hydrogen, methane, and other valuable products. Enzymatic fuel cells (EFCs) gathered with MFCs become a major part of BES. Likewise, MFCs are classified in other BES applications, which are microbial electrochemical cells (MECs), microbial desalination cells (MDCs), and microbial solar cells (MSCs) (Pant et al. 2012). Therefore, the term MFC is appropriately used to elucidate this study in order to simplify the comprehensive understanding, as depicted in **Figure 2.8**.

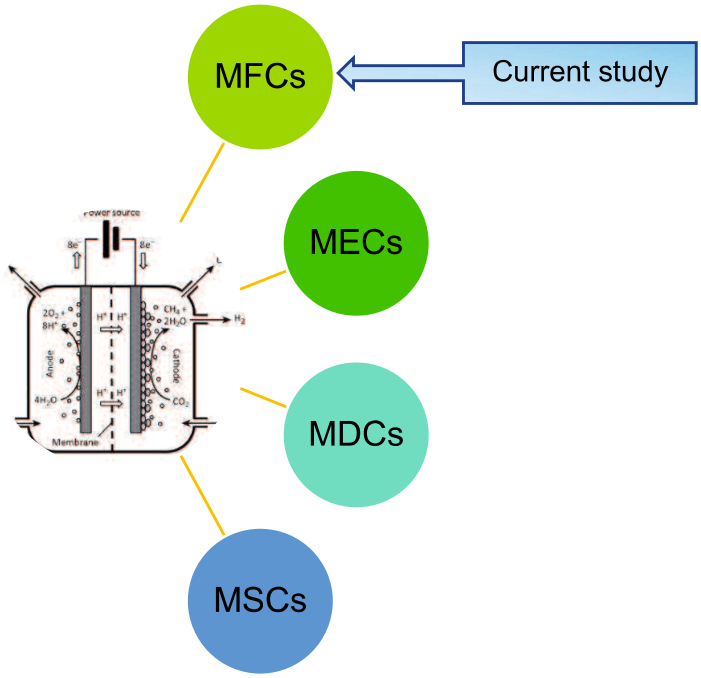

Figure 2.8 Bioelectrochemical systems (BESs) in the application. A figure is adopted from (Geppert et al. 2016).

2.4.2. Basic principle of MFC

MFC, an emerging technology nowadays, has the possibility to convert organic substances releasing chemical energy to direct electricity involving electroactive bacteria (EAB) as biocatalysts. The MFC is emerging as a versatile renewable energy technology because of its multidimensional applications (Kumar et al. 2018). In addition, MFCs have the potential for organic and inorganic waste treatment concomitant with power generation (Tan et al. 2021). Likewise, MFCs have attracted considerable attention due to their versatility in their applications in wastewater treatment, power generation, toxic pollutant removal, environmental monitoring sensors, and more (Paucar and Sato 2021). Furthermore, MFCs are preferred because they are clean, safe, economically viable, and environmentally beneficial technologies (Din et al. 2021). MFC consists of electrodes (anode and cathode), inoculum, and substrate as anolyte in one chamber as a single chamber microbial fuel cell (SCMFC). Whereas a double chamber microbial fuel cell (DCMFC) consists of all components in SCMFC, assisted with separator and catholyte, separated in two chambers.

In **Figure 2.9**, MFCs carry out the organic substances, from complex or high strength levels to single or low strength levels, converted to simple strength levels by involving microorganisms as biocatalysts and generating electricity simultaneously. The anode zone plays an important role in converting organic substances into carbon dioxide (CO_2), hydrogen ions (H^+) as protons, and electrons (e^-). The condition of the anode zone could be either anoxic or obligate anaerobic. In addition, the main process of its zone is oxidation which is hydrolysis. While the cathode zone is primarily a reduction process, which changes the protons produced from the anode zone by combining oxygen into water.

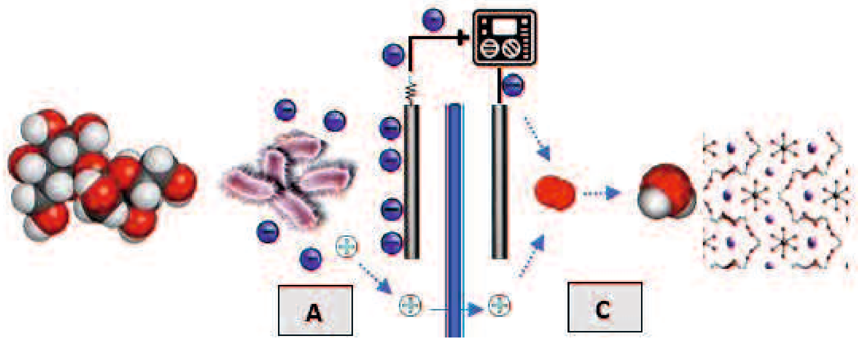

Figure 2.9 Schematics of the principle of a double-chambered MFC. Adopted from elsewhere. A = Anode zone; C = Cathode zone.

The complete process can be represented as [1] and [2a & 2b] for sucrose, [3] and [4a & 4b] for acetate, and [5] and [6a & 6b] for other complex or single substrates.

Sucrose

Anode half-cell reaction: $C_{12}H_{22}O_{11} + 13H_2O \text{ --> } 12CO_2 + 48e^- + 48H^+$ (1)

Cathode half-cell reaction: $12O_2 + 48e^- + 48H^+ \text{ --> } 24H_2O$ (Acidic conditions) (2a)

Cathode half-cell reaction: $12O_2 + 24H_2O + 48e^- \text{ --> } 48OH^-$ (Alkaline conditions) (2b)

Acetate

Anode half-cell reaction: $C_2H_3O_2Na + 2H_2O \text{ --> } 2CO_2 + Na^+ + 7e^- + 7H^+$ (3)

Cathode half-cell reaction: $1.75O_2 + 7e^- + 7H^+ \text{ --> } 3.5H_2O$ (Acidic conditions) (4a)

Cathode half-cell reaction: $1.75O_2 + 3.5H_2O + 7e^- \text{ --> } 7OH^-$ (Alkaline conditions) (4b)

Other complex or single substrates

Anode half-cell reaction: $C_xH_yO_z + nH_2O \text{ --> } nCO_2 + ne^- + nH^+$ (5)

Cathode half-cell reaction: $nO_2 + ne^- + nH^+ \text{ --> } nH_2O$ (Acidic conditions) (6a)

Cathode half-cell reaction: $nO_2 + nH_2O + ne^- \text{ --> } nOH^-$ (Alkaline conditions) (6b) where x, y, and z are the number of atoms; n is the number of molecules. This study utilizes sucrose and acetate in AC-SCMFC and sucrose in DAC-DCMFC. The difference potential between anode and cathode stimulates electricity generation. Logan (2009) described the potential of microbial fuel cells (MFCs) compared to reactors with defined potentials in which bacteria contribute to provoking potential energy from 100 mV to 820 mV through the utilization of acetate as a substrate; furthermore, the presence of an anode in the MFC contributes -200 mV, and at the same time, a cathode contributes 250 mV; therefore, the potential energy (ΔE) is calculated as 450 mV which means the voltage displayed on the data logger or multimeter. Compared to the electrochemical reactor, ΔE is measured at 820 mV with an anode potential of 520 mV and an acetate potential of -300 mV.

2.4.3. Microbes

The term microorganism in MFC is known as electroactive bacteria (EAB) or exoelectrogenic bacteria (EEB) or electricigens or electrogenic bacteria (EB), or electrotrophic microbial consortia (EMC), which can degrade organic substances, produce electrons outside of their body structure, and subsequently transfer the electron to an anode using their special nanowire. This study uses the EAB term to elucidate the name of MFC bacteria. Not all EABs can transfer the electrons directly to an anode, but some of them need a mediator, which has a function to favour bacteria transferring the electrons chemically. Therefore, EAB can be divided into two kinds: EAB without a mediator or mediatorless, and EAB with a mediator. General EABs, genus scale, utilized in many MFC experiments are depicted in **Figure 2.10**.

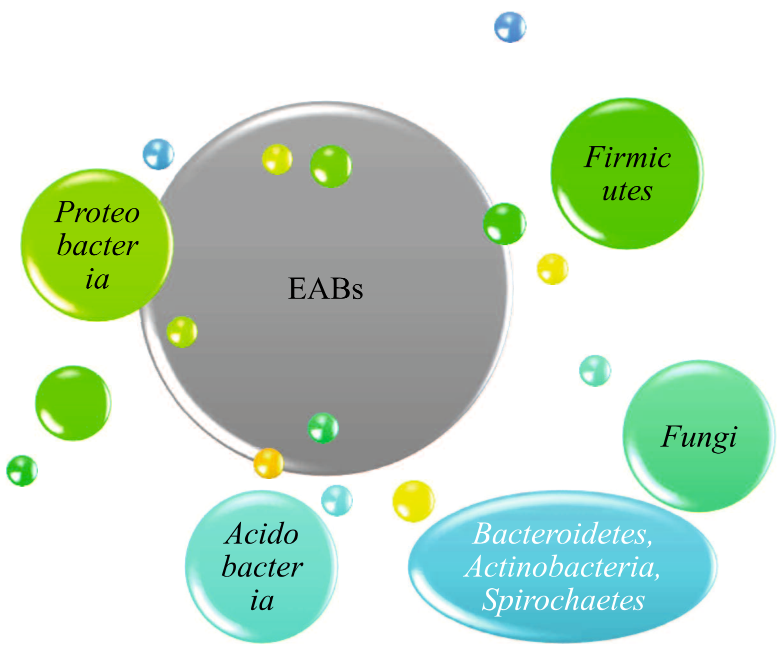

Figure 2.10 MFC bacteria. Reported elsewhere.

Many EABs play a role in many types of MFC studies, which can be mainly divided into Proteobacteria, Firmicutes, Acidobacteria, Bacilli, and Fungi, as reported elsewhere. However, only Proteobacteria and Firmicutes are mostly available in anodic biofilm and anolyte/substrate. The most well-known Proteobacteria genera are alfa-, beta-, gamma-, and delta-Proteobacteria. In addition, gamma- and delta-Proteobacteria have been used widely in such related areas, particularly *Shewanella sp.* and *Geobacter sp.,* which utilize iron as their electron acceptor. Whereas Acidobacteria, Bacilli, and Fungi rarely exist in some MFC studies, they were still taken into account.

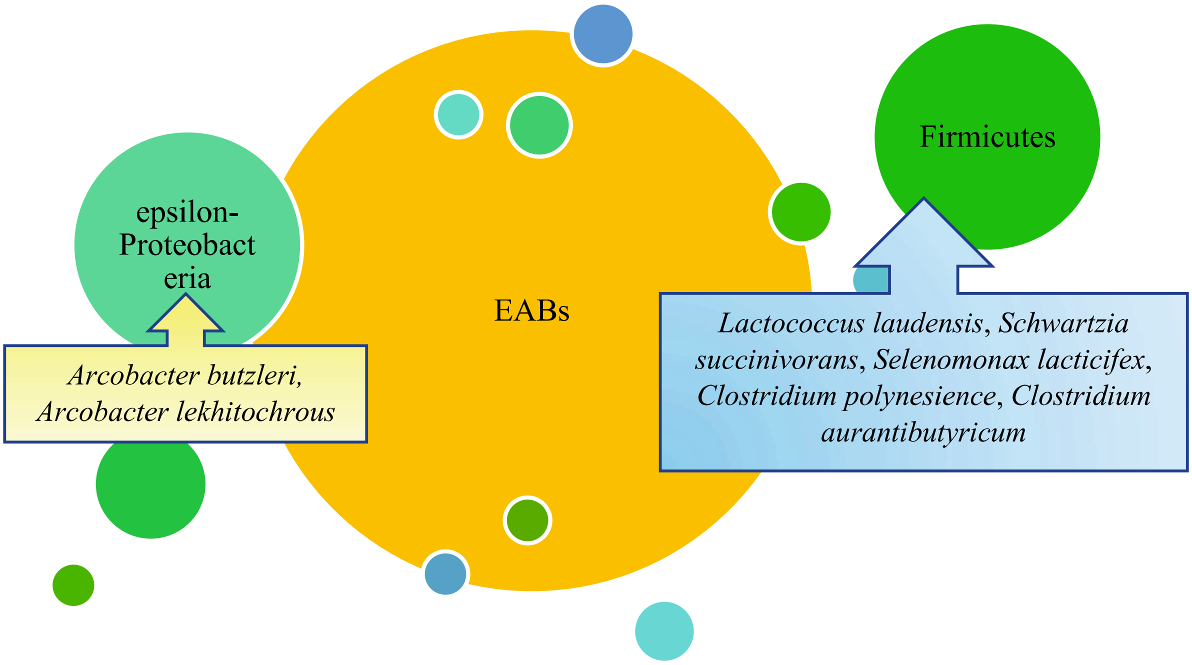

Figure 2.11 The current study uses MFC bacteria from epsilon-Proteobacteria and Firmicutes as a substrate-based mixed culture

This study revealed epsilon-Proteobacteria and Firmicutes, which are *Arcobacter butzleri* and *Arcobacter lekhitochrous*, classified as epsilon-Proteobacteria, and *Lactococcus laudensis*, *Schwartzia succinivorans*, *Selenomonax lacticifex*, *Clostridium polynesience*, and *Clostridium aurantibutyricum*, identified as Firmicutes, as described in **Figure 2.11**. A mixed culture with 90% of *Arcobacter butzleri* performed a maximal power density of 296 mW/L (Fedorovich et al. 2009). In addition, the complete genome of *Arcobacter butzleri ED-1* and *Arcobacter sp. Strain L*, both were isolated and characterized from the anode biofilm and the liquid phase of a microbial fuel cell, respectively (Toh et al. 2011). Furthermore, the mixed culture consisting of microbial consortia, Arcobacter, Aeromonas, Pseudomonas, Acinetobacter, Cloacibacterium, and *Shewanella sp.,* took performing in phenolic degradation of 41% and high current density of 156 mA/m^2 (Hassan et al. 2018). Moreover, Szydlowski et al. (2020) found that engineered *Arcobacter butzleri* known mutant *Arcobacter butzleri*, as a pure culture, conditioned by acetate and lactate substrates, generated average current densities of 81-82 mA/m^2. Therefore, Arcobacter sp., particularly, can be exclusively classified as EAB.

Similar to *Arcobacter butzleri* above mentioned that *Arcobacter lekhitochrous* has the same function of electron-producing and transferring active bacteria (Fedorovich et al. 2009; Toh et al. 2011; Pereira-Medrano et al. 2013; Szydlowski et al. 2020). Likewise, *Clostridium polynesiense* and *Clostridium aurantibutyricum*, a part of the Clostridium group, are particularly defined as firmicutes that are stated to be one of EABs, having a similar function with "-proteobacteria. At the same time, *Lactococcus laudensis*, *Schwartzia succinivorans*, and *Selenomonas lacticifex* are synergistic as a catalyst to convert organic substances to electrons. *Schwartzia sp.* and *Selemonas sp.*, specified as anaerobic, Gram-negative, and non-spore-forming bacteria, are the most closely related genera (Van Gylswyk, Hippe, and Rainey 1997). Lactococcus sp. is a Gram-positive bacteria that utilize the soluble redox mediators taken by Gram-negative bacteria (Pham et al. 2008; Rabaey et al. 2005). Since they are also characterized as a rare utilization in many MFC studies; therefore, this study contributes to better insight into comprehending the influence of epsilon-Proteobacteria and Firmicutes as a mixed culture on MFCs.

2.4.4. Microbial fuel cell designs

MFC is principally designed in a simple way, scalable from bench scale to full scale, easy to use, and at a lower cost. Koffi and Okabe (2020) noticed that MFCs should be inexpensive, scalable, and watertight (withstand high water pressure) and produce good effluent water quality (meet effluent standards so that there is no need for post-treatment) and usable power. The basic designs of MFC consist of single, double, and stack/multiple chambers. In detail, as depicted in **Figure 2.12**, **Figure 2.13**, **Figure 2.14**, **Figure 2.15**, and **Figure 2.16**, Flimban et al. (2019) classify single, double, and stack chambers into several types, which are single: with and without separators; double: common double chamber, mini square-shaped compact flat plate MFC (FPMFC) called cubed chamber, and upflow MFC (UMFC); and stack: bipolar electrode, horizontal, and vertical MFC stacks. In addition, Rahimnejad, Asghary, and Fallah (2020) appended an H-shaped as the MFC series complemented those mentioned above.

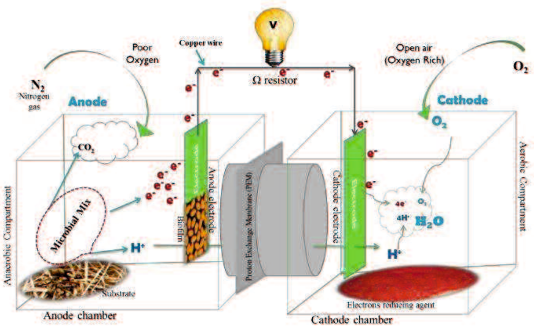

Figure 2.12 DCMFC
Credited to: Flimban et al. (2019).

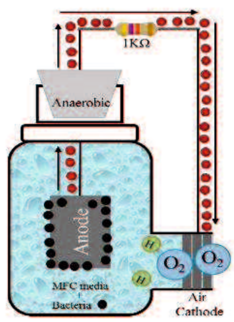

Figure 2.13 SCMFC
Credited to: Flimban et al. (2019).

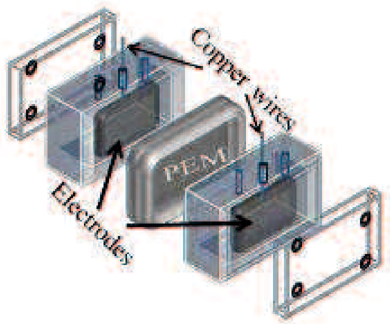

Figure 2.14 Flat plate MFC (FPMFC)
Credited to: Flimban et al. (2019).

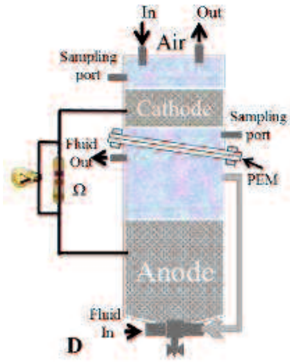

Figure 2.15 Upflow MFC (UMFC)
Credited to: Flimban et al. (2019).

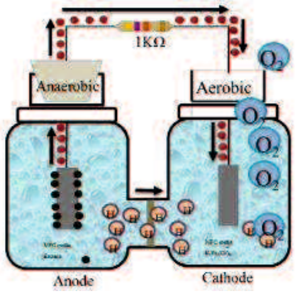

Figure 2.16 H-shaped MFC
Credited to: Flimban et al. (2019).

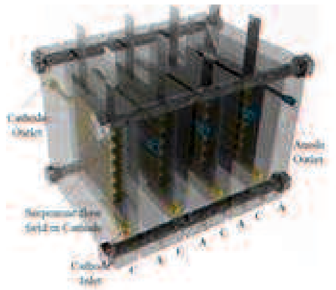

Figure 2.17 Stacked MFC
Credited to: Zhang et al. (2017).

Currently, stacked MFCs, as described in **Figure 2.17**, perform high performance in power generation compared to SCMFCs and DCMFCs; however, power overshoot occurs in their application. This series is concerned with generating electricity rather than increasing the organic removal either in large-scale MFC or in the integrated MFC-wastewater treatment. Therefore, this research can be a bridge to resolve and contribute to developing a multi-chamber MFC.

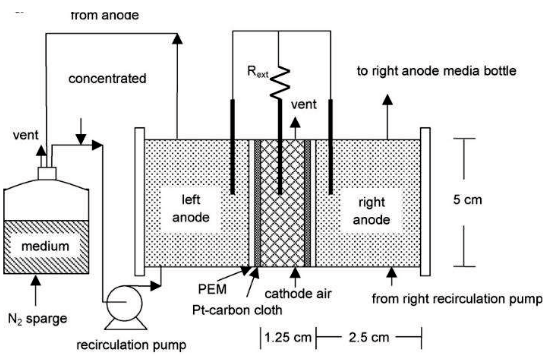

Figure 2.18 Dual anode chambered MFC
Credited to: Kim et al. (2011).

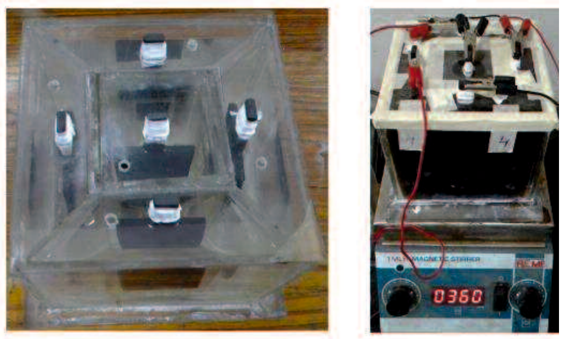

Figure 2.19 Multi-chamber MFC
Credited to: Samsudeen et al. (2015).

Figure 2.12, **Figure 2.17**, **Figure 2.18**, and **Figure 2.19** trigger this study, comprehensively understanding the simultaneous performance of organic removal and power generation.

Concerning this sub-sub chapter 2.4.4, thus observing the nearest references is conducted in order to be a benchmark analysis in chapters III and IV. Several papers closely related to this research were observed carefully, starting from screening to selecting articles related to the closest research. Considering to get easier understandable related in this study, thus this literature review used artificial intelligence web-based searches, which are connected papers (https://www.connectedpapers.com/) and research rabbit (https://www.researchrabbit.ai/). The screening method is depicted in **Figure 2.20**, **Figure 2.21**, and **Table 2.1**.

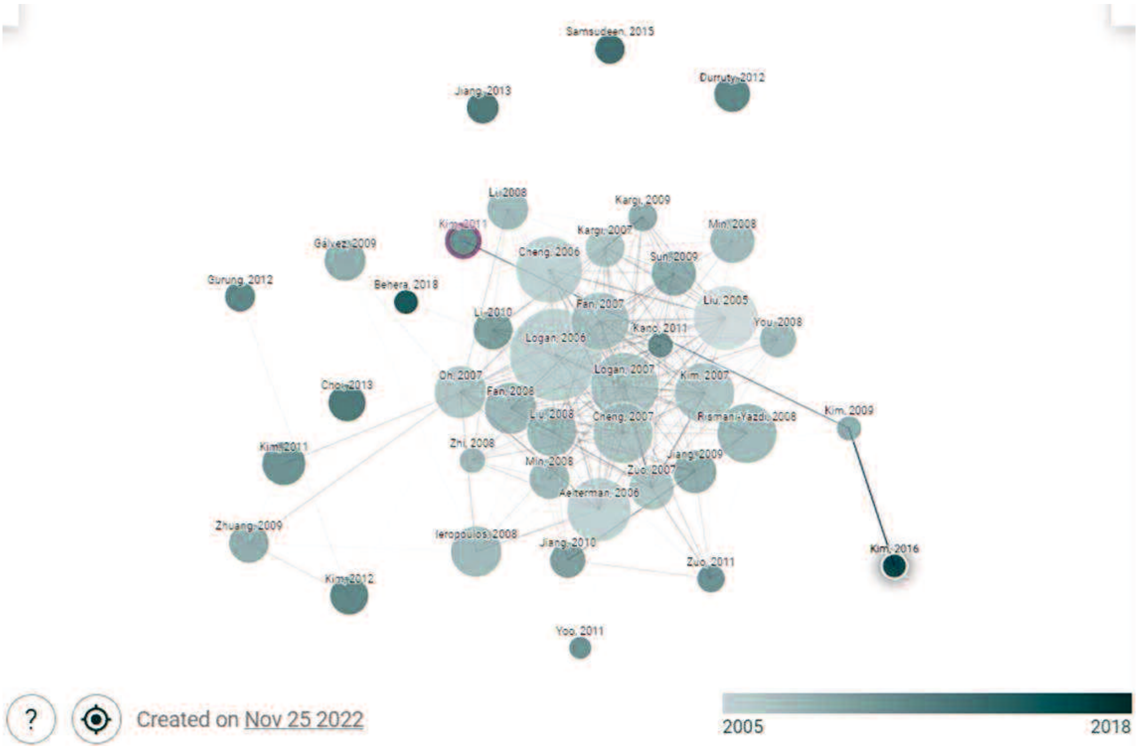

Figure 2.20 Network citations of dual anode chamber MFC by www.connectedpapers.com

Figure 2.20 above describes the interrelation of several articles with a similar object in the study. In the example, this study focuses on the multi-chamber anode of MFC with the keyword "dual anode chamber MFC". The older article was published in 2005, and the newest one was published in 2018. The exact article with the mentioned keyword was studied by Kim *et al.*, (2011), who cited the previous article with the biggest attention on MFC investigated by Logan *et al.* (2006). However, this figure is not clearly understood due to more networking in the papers with a general study, not specific research. Therefore, it needs other alternatives to describe the appropriate reports correlation.

Furthermore, the other artificially intelligent web-based is performed through research rabbit (www.researchrabbitapp.com), which generates a more crystal-clear understood the papers related to this study. **Figure 2.21** below-mentioned depicts the focus study in dual anode chamber MFC; thus, it is used for further literature review analysis.

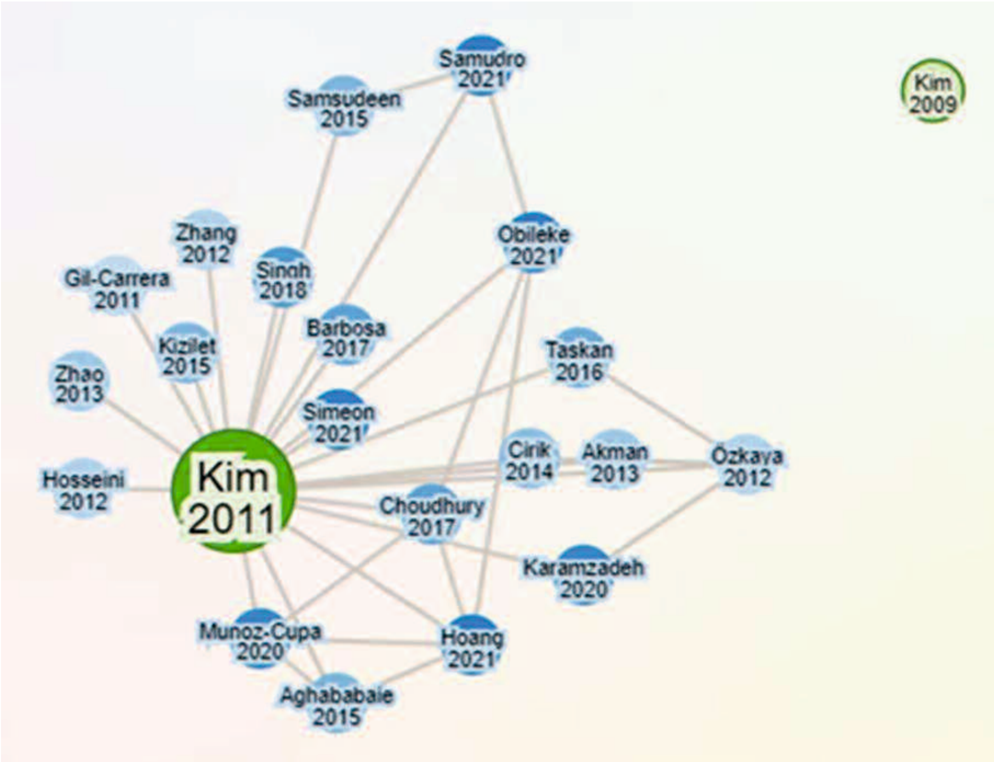

Figure 2.21 Network citations of dual anode chamber MFC by www.researchrabbitapp.com

Table 2.1 Screening method for literature review through artificial intelligence web-based with the main keyword of dual anode chamber MFC

Category	Reference	Type of reference	Type of MFCs
Closely related	An analysis of the performance of an anaerobic dual anode-chambered microbial fuel cell (Kim *et al.*, 2011)	Research	Multi-chamber anode DCMFCs
	Performance investigation of multi-chamber microbial fuel cell: An alternative approach for scale-up system (Samsudeen *et al.*, 2015)	Research	Multi-chamber anode DCMFCs
	Enhancement of power generation and organic removal in double anode chamber designed dual-	Research	Multi-chamber anode DCMFCs

Category	Reference	Type of reference	Type of MFCs
	chamber microbial fuel cell (Samudro, Imai and Hung, 2021)		
No closely related but referenced	Optimizing the electrode size and arrangement in a microbial electrolysis cell (Gil-Carrera *et al.*, 2011)	Research	Membrane-less flat-plate microbial electrolysis cell (MEC)
	Iron tetrasulfophthalocyanine functionalized graphene as a platinum-free cathodic catalyst for efficient oxygen reduction in microbial fuel cells (Zhang *et al.*, 2012)	Research	Dual-chamber microbial fuel cells
	Bioelectricity production using a new electrode in a microbial fuel cell (Ozkaya *et al.*, 2012)	Research	Dual-chamber microbial fuel cells
	Microbial fuel cells-air-cathode electrodes (Zhao *et al.*, 2018)	Research	Air-cathode microbial fuel cells
	A dual-chambered microbial fuel cell with Ti/nano-TiO2/Pd nano-structure cathode (Hosseini and Ahadzadeh, 2012)	Research	Dual-chamber microbial fuel cells
	Bioelectricity generation in continuously-fed microbial fuel cell: Effects of anode electrode material and hydraulic retention time (Akman *et al.*, 2013)	Research	Dual-chamber microbial fuel cells
	Optimization of bioelectricity generation in fed-batch microbial fuel cell: effect of electrode material, initial substrate concentration, and cycle time (Cirik, 2014)	Research	Dual-chamber microbial fuel cells
	Biocathode application in microbial fuel cells: Organic matter removal and denitrification (Kizilet *et al.*, 2015)	Research	Dual-chamber microbial fuel cells

Category	Reference	Type of reference	Type of MFCs
	Effective factors on the performance of microbial fuel cells in wastewater treatment:a review (Aghababaie *et al.*, 2015)	Review paper	
	Comprehensive evaluation of two different inoculums in MFC with a new tin-coated copper mesh anode electrode for producing electricity from a cottonseed oil industry effluent (Taskan, Ozkaya and Hasar, 2015)	Research	Dual-chamber microbial fuel cells
	Investigating bacterial community changes and organic substrate degradation in microbial fuel cells operating on real human urine (Barbosa *et al.*, 2017)	Research	Parallel dual-chamber microbial fuel cells
	Performance improvement of microbial fuel cells for wastewater treatment along with value addition: A review on past achievements and recent perspectives (Choudhury *et al.*, 2017)	Review paper	
	Bio energy production using carbon based electrodes in double and single chamber microbial fuel cells: A review (Singh, 2018)	Review paper	
	Modelling the influence of substrate concentration, anode electrode surface area and external resistance in a start-up on the performance of microbial fuel cell (Karamzadeh *et al.*, 2020)	Research	Single chamber microbial fuel cell
	An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production (Munoz-Cupa *et al.*, 2021)	Review paper	

Category	Reference	Type of reference	Type of MFCs
	Microbial fuel cells for bioelectricity production from waste as sustainable prospect of future energy sector (Hoang *et al.*, 2022)	Review paper	
	Influence of electrode spacing and fed-batch operation on the maximum performance trend of a soil microbial fuel cell (Simeon and Freitag, 2022)	Research	Single chamber microbial fuel cell
	Microbial fuel cells, a renewable energy technology for bio-electricity generation: A mini-review (Obileke *et al.*, 2021)	Review paper	

From **Table 2.1** above-mentioned, then, the screening method for the literature review is focused on Kim *et al.*, (2011) afterwards. In addition, the closely related category shows 100% of the research paper type; whereas no closely related category offers 67% of the research paper type in which the review paper notices 33%. This study has had great attention in the MFC research development until now and has been supported by the review paper on MFC improvement.

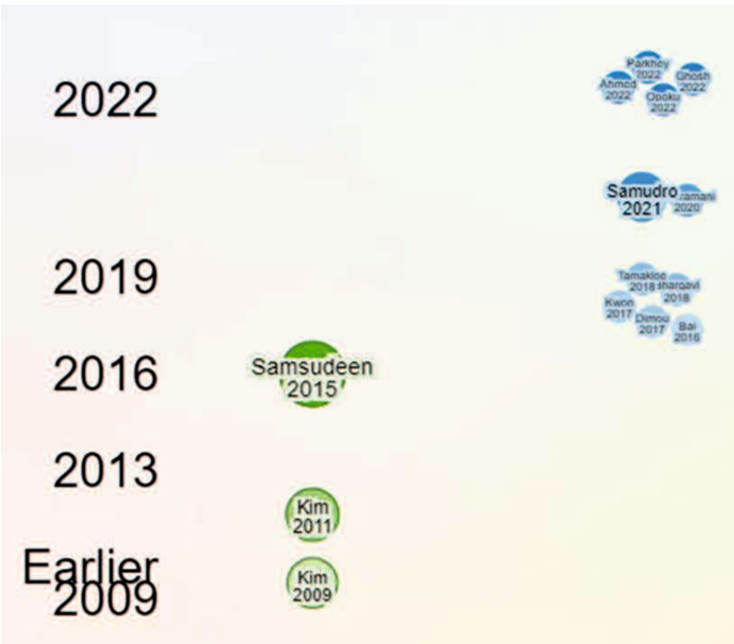

Figure 2.22 Network citations of dual anode chamber MFC by www.researchrabbitapp.com focused on Kim *et al.* (2011)

Table 2.2 Screening method for literature review through artificial intelligence web-based with the main keyword of dual anode chamber MFC following the study of Kim *et al.* (2011)

Category	Reference	Type of reference	Type of MFCs
Closely related	An analysis of the performance of an anaerobic dual anode-chambered microbial fuel cell (Kim *et al.*, 2011)	Research	Multi-chamber anode DCMFCs
	Performance investigation of multi-chamber microbial fuel cell: An alternative approach for scale up system (Samsudeen *et al.*, 2015)	Research	Multi-chamber anode DCMFCs
	Enhancement of power generation and organic removal in double anode chamber designed dual-chamber microbial fuel cell (Samudro, Imai and Hung, 2021)	Research	Multi-chamber anode DCMFCs
No closely related but referenced	Advanced nanomaterials for the design and construction of anode for microbial fuel cell (Bai, Zhou and Gu, 2016)	Research	Unknown
	Performance of pilot scale plug flow microbial fuel cell for sustainable wastewater treatment and energy recovery (Dimou, 2017)	Research	Air-cathode multi-electrode single chamber microbial fuel cell
	Comparison of spacer-less and spacer-filled reverse electrodialysis (Kwon *et al.*, 2017)	Research	Stack microbial fuel cell
	Microbial fuel cells: recent developments in design and materials (Bhargavi, Venu and Renganathan, 2018)	Review paper	
	PEM-less microbial fuel cells (Tamakloe, 2018)	Book chapter	
	Performance of cobalt oxide/carbon cloth composite electrode in energy generation from dairy wastewater using microbial fuel cells	Research	Dual-chamber microbial fuel cell

Category	Reference	Type of reference	Type of MFCs
	(Veeramani, Rajangam and Nagendran, 2020)		
	Insights into the development of microbial fuel cells for generating biohydrogen, bioelectricity, and treating wastewater (Ahmed *et al.*, 2022)	Review paper	
	Scale-up of bioelectrochemical systems: stacking strategies and the road ahead (Parkhey, 2022)	Review paper	
	Impact of wastewater volume on cathode environment of the multi-anode shared cathode and standard single anode/cathode microbial fuel cells (Opoku *et al.*, 2022)	Research	Multi-electrode dual chamber microbial fuel cell
	Metabolic engineering and synthetic biology key players for improving efficacy of microbial fuel cell technology (Ghosh *et al*., 2022)	Book chapter	

Considering the focus study of Kim *et al.* (2011) and Samsudeen *et al.* (2015), this study is compared to know their differences for depth analysis purposes. Their comparison is shown in **Table 2.3**.

In addition, the closely related category shows 100% of the research paper type; whereas no closely related category offers 50% of the research paper type in which the review papers and book chapters notice 33% and 17%, respectively. Similar to the first screening, this study provides close attention to MFC improvement.

Table 2.3 Comparison between this study with the most related-found reference

Factors	(Kim *et al.*, 2011)	(Samsudeen *et al.*, 2015)	(Samudro, Imai and Hung, 2021)
Design and configuration	Incorporating two anode chambers flanking a shared air cathode chamber	Consisted of four anodes and a cathode chamber separated by a PEM	Separated anode chambers into two parts in a dual anode

Factors	(Kim *et al.*, 2011)	(Samsudeen *et al.*, 2015)	(Samudro, Imai and Hung, 2021)
			chamber microbial fuel cell
The working volume (mL)	15 mL @ 2 units of anodic chamber	210 mL @ 4 units of anodic chamber	500 mL @ 2 units of anodic chamber
Retention time	6.2 min	-	24 h
Mode	Continuous	Batch	Fed-batch
Components	**Anode**: Graphite rod (d = 0.6 cm, h = 5 cm) **Cathode**: The Pt-impregnated cathodes (d = 0.6 cm, h = 5 cm)	**Anode**: Plain graphite flat plates (4 x 6) cm **Cathode**: (4 x 6) cm • Flat plate • Cylinder • Shaped graphite	**Anode**: GNPs-SSM 30 mesh (4.9 x 4.9) cm **Cathode**: (4.9 x 4.9) cm • GNPs:PTFE-SSM 30 mesh • GNPs:PU-SSM 30 mesh
Recommendations	• The feed flow rate = 5 ml/min • Lactate concentration = 10 mmol/L	• The distillery wastewater concentration = 8720 mg COD/L • Catholyte concentration = 100 mM	• Medium OLR achieved the maximum organic removal ranging from 60% to 80% • The second anode chamber achieved higher performance in power production • The type 3 reactor provides a better performance
Focused performance	Power generation, lactate removal	Power generation	Power generation, COD removal
Achievements	**Power generation**:	**Power generation**:	**Power generation**: • PD = 866 mW/m^2

Factors	(Kim *et al.*, 2011)	(Samsudeen *et al.*, 2015)	(Samudro, Imai and Hung, 2021)
	• PD = 24 W/m^3 (458 mW/m^2) • I = 1.69 mA (at 100Ω) **Lactate removal**: $C_3H_6O_3$r = 55% (in 1 d)	• PD = 135.4 mW/m^2 (1.6 W/m^3) • CD = 368 mA/m^2 **COD removal**: No identified information	• PD = 5.15 W/m^3 **COD removal**: CODr max = 60 – 80% (in 1 d)

Table 2.3 shows a similar configuration using more than one anode chamber connected to one cathode chamber; however, the position of the anode chamber to the cathode chamber was different. The working volume, retention time, mode, and component also indicated differences. Nevertheless, the focused performance has quite similar between Kim *et al.* (2011) and Samudro, Imai and Hung (2021) for simultaneous performance achievements. Power generation in surface power density of Samudro, Imai and Hung (2021) study was noticed better compared to Kim *et al.* (2011) and Samsudeen *et al.* (2015); but, in the volumetric power density of Kim *et al.* (2011) study showed better than Samsudeen *et al.* (2015) and Samudro, Imai and Hung (2021). Furthermore, Samudro, Imai and Hung (2021) study on organic removal outperformed rather than Kim *et al.* (2011) study. Samsudeen *et al.* (2015) study used batch mode in their operation, and there was no information about organic removal as well. In surface power density, Samudro, Imai and Hung (2021) study achieved 1.9 times that of Kim *et al.* (2011) with a similar operation mode. On the other hand, the volumetric power density of Kim *et al.* (2011) study reached 4.7 times that of Samudro, G. et al. (2021). Due to many references based on the surface power density for power generation; hence, surface power density is used as the main reference of power generation.

2.4.5. Factors affecting microbial fuel cells

Several factors, which are electrode material, substrate, membrane, microbes, applied voltage, microbial inoculation, physico-chemical characteristics of electrodes, organic loading rate (OLR), and hydraulic retention time (HRT), influence the performance of MFC. Saravanan et al. (2020) classified the factors affecting of MFCs into two factors which are operational parameters and effective factors. In detail, the operational parameters consist of applied voltage, microbial inoculation, physico-chemical characteristics of electrodes, OLR, and HRT; meanwhile, the effective factors consist of electrode material, substrate, membrane, and microbes. Moreover, Samudro, Imai, and Hung (2021) mentioned the factors affected MFC performance, such as parameters' control (pH, and temperature) (Igboamalu et al. 2019), anolyte (Potrykus et al. 2021), catholyte (Lawson et al. 2020; Rossi and Logan 2020; Waheeb and Al-Alalawy 2020; Gajda et al. 2020), inoculum (Nath and Ghangrekar 2020; Ren et al. 2021), anode (Rajesh, Noori, and Ghangrekar 2020; Jingyu et al. 2020), cathode (Chakraborty et al. 2020; Aysla Costa De Oliveira et al. 2020), electrode spacing (You, Greenman, and Ieropoulos 2018; Singh and Kaushik 2021), separator (Flimban et al. 2020; Shabani et al. 2020), flowrate (Ieropoulos, Winfield, and Greenman 2010; Krieg et al. 2018), retention time (Santos, Barros, and Linares 2017), wiring system (Tominaga, Ototani, and Darmawan 2020), design/construction (Miller et al. 2019), and configuration (Aelterman et al. 2006; Clauwaert et al. 2008; Nandy and Kundu 2018). Therefore, the main effects of MFC can be generally defined: electrode material (anode and cathode), anolyte as substrate, inoculum, and separator, as the effective factors, and retention time as the operational parameters.

In this study, the primary factors are considered by using substrate and retention time as the independent variables. As reported elsewhere, substrate, either their type or their concentration, has a significant correlation between organic removal and power generation; meanwhile, retention time (RT) is a critical factor in designing the appropriate volume of MFC and substantially correlating in the process, i.e., kinetics, of the substrate. Therefore, the selection of the independent variables is mainly based on the significance of the variables, particularly in organic removal and power generation. Whereas the electrode material, inoculum, and separator are used as the dependent variables. Two types of saccharides consisting of monosaccharides (acetate), which are represented as highly oxidized, and disaccharides (sucrose), and are defined as oxidizable, are utilized because they are extensively used in various types of MFC studies and can be represented as part of real wastewater that can be treated properly (G. Samudro, Imai, and Reungsang 2022). On the other hand, RT plays an important role to be used in designing the MFC afterwards. It is described and concluded in **Figure 2.23** and **Figure 2.24**.

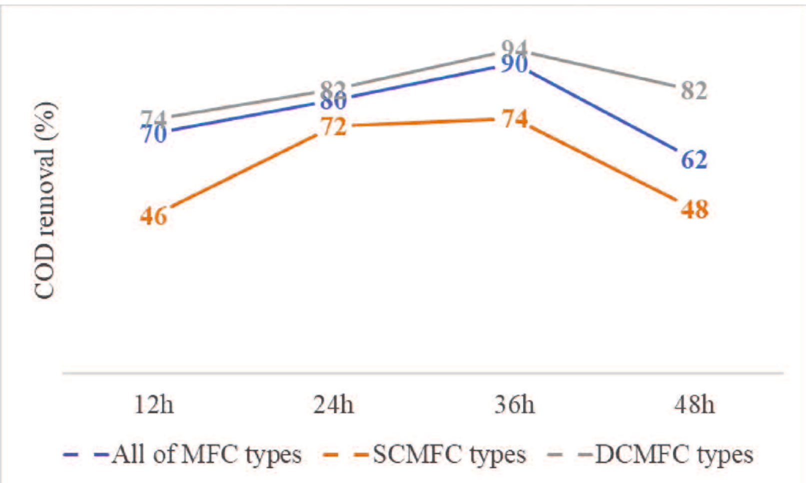

Figure 2.23 Organic removal performance of various MFC types. Reported elsewhere.

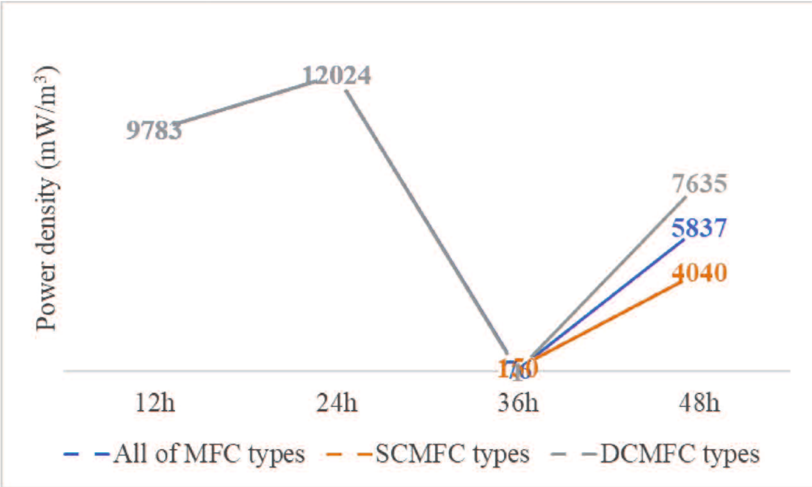

Figure 2.24 Power generation performance of various MFC types. Reported elsewhere.

Figure 2.23 and **Figure 2.24** above are collected by grouping three types of MFC: SCMFC, DCMFC, and stack MFC, and various substrate types and concentrations. Based on the literature review, RT of more than 24 h was better in organic removal; meanwhile, it was worse in power generation. Therefore, the appropriate RT must be determined in advance in order to obtain optimal MFC performance both in organic removal and power generation. In this study, the optimum RT with the difference in substrate types and concentrations is determined through performing AC-SCMFC, and subsequently, the RT is used to design DAC-DCMFC to achieve high performance.

2.4.6. Applications of microbial fuel cells

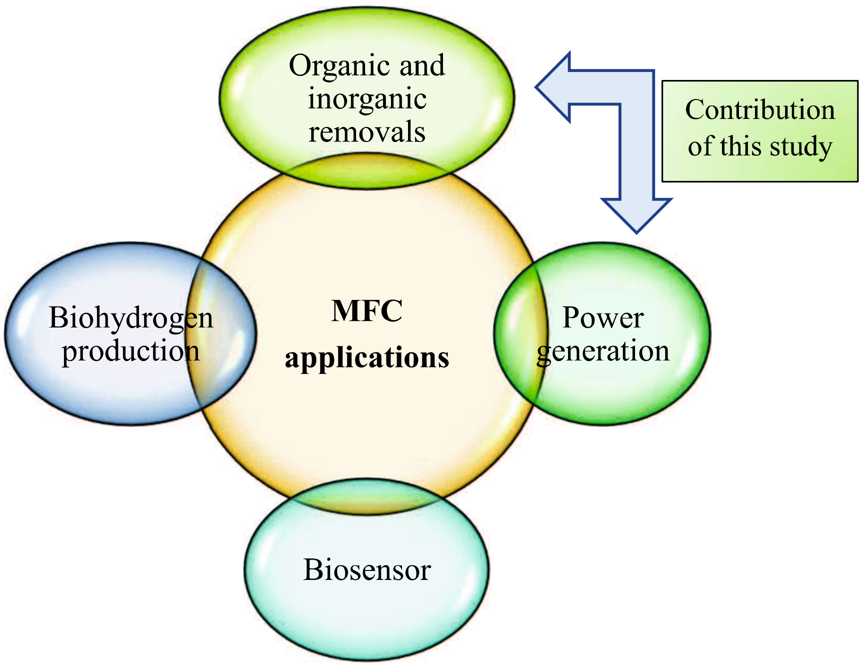

Figure 2.25 Several MFC applications. As reported elsewhere.

Currently, MFC has been developed for integrating wastewater treatment and power harvesting simultaneously and for other particular purposes in environmental monitoring as a biosensor and biohydrogen production. Regarding MFC applications are described in **Figure 2.25**. Goswami and Mishra (2017) mentioned that MFCs could be used for biosensors, wastewater treatment, and biohydrogen production. As a biosensor MFC, Tardy et al. (2021) noticed that MFCs could be used as automatic on-site BOD sensors for real wastewater samples. Whereas for biohydrogen production, MFC is assisted with a biohydrogen reactor to increase energy yield using raw cheese whey as a substrate (Wenzel et al. 2017). Some different principles appear pertaining to their goals: producing simultaneous organic removal and power generation and biosensors; thus, an anode chamber should be set either in anaerobic or anoxic conditions and keep the cathode chamber in aerobic conditions. For biohydrogen purposes, the cathode condition is designated as an obligate anaerobic. However, some limitations exist in the context of practical wastewater treatment due to a lack of insight into adjusting the factors that affect MFC.

2.4.7. Limitations

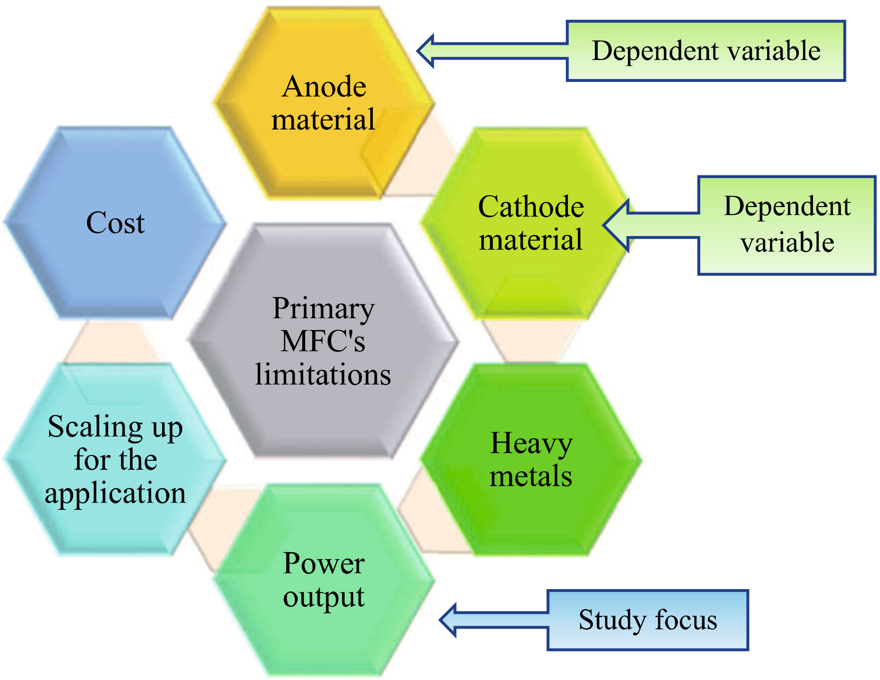

Figure 2.26 Primary MFC’s limitations

Findings from 2017-2022 show that the limitations of MFC occur due to several factors, such as anode and cathode materials, heavy metals, cost, scaling up for the application, power output, parts and materials, substrate type, methods, remedial achievement, aromatic hydrocarbon, oil and grease, sludge, biosensor, anode surface, catalyst, and commercial aspect. In addition, anode and cathode materials, heavy metals, power output, scaling up for the application, and cost have been concerned as the main limitations, as depicted in **Figure 2.26**. Whereas the other factor's limitations could be developed in further research. Therefore, the emerging concerned aspect in this study was focused on scaling up for the application. Since it has been a point of view in many studies according to match the realization of MFC in the field, which should be efficient and effective, particularly in terms of design and cost.

2.5. Air-Cathode Single-Chamber MFC (AC-SCMFC) for the Determination of the Substantial Factors

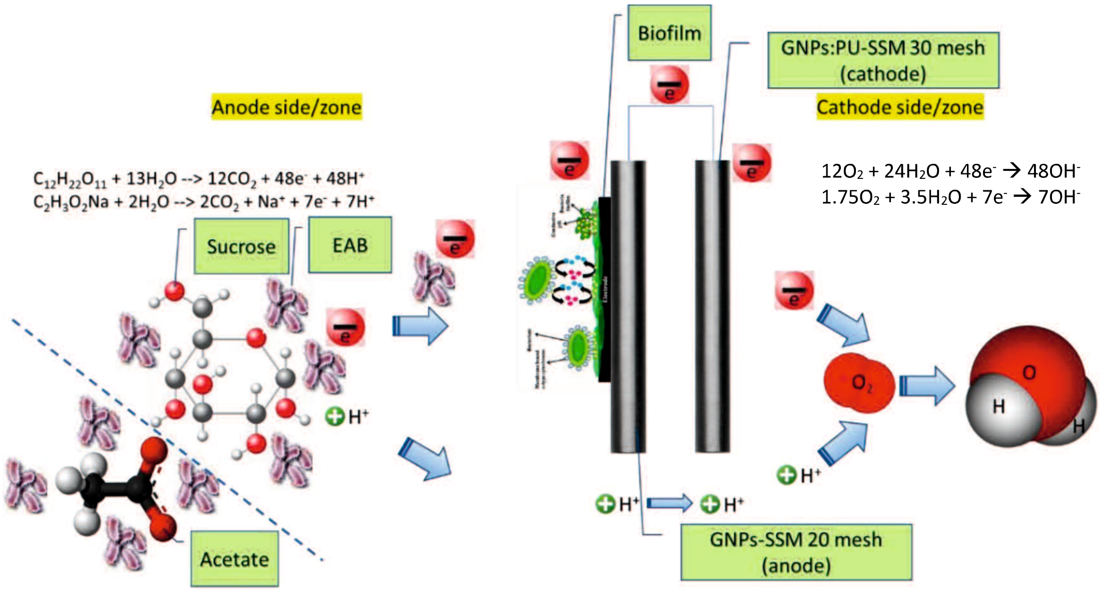

Figure 2.27 The basic design concept of the current experiment in AC-SCMFC

Electrode (anode and cathode), separator, microbial community, substrate (type and concentration/loading) as anolyte, catholyte, and operating mode, found in many references, are the critical point of factors affecting the MFCs' performance. Some of them, the anode, microbial community, substrate, and operating mode, are very influential. However, the determination of their best and optimum condition is still incomprehensible. There are many different methods with different results, even under the same situation. Therefore, relevant results, even closely, are highly expected by using the suited method. AC-SCMFC has become appropriately used to identify and know the optimum condition, even using different techniques. A new approach employed in the batch process in MFC studies could make it easier to obtain the optimum RT (Thompson, Runge, and Dunne 2019; von Sperling, Verbyla, and Oliveira 2020). The batch process is easier to explain the significant patterns depicted in the substrate consumption behaviour as a function of time by microorganisms. In detail, the comprehensive explanation is shown in Chapter III about the determination of optimum retention time in an air–cathode single chamber microbial fuel cell batch-mode reactor by comparing different substrate types and concentrations, which is published in Process Safety and Environmental Protection journal (https://www.sciencedirect.com/science/article/pii/S0957582022003305). G. Samudro, Imai, and Reungsang (2022) briefly figured out the conceptualization in **Figure 2.27** and **Figure 2.28**.

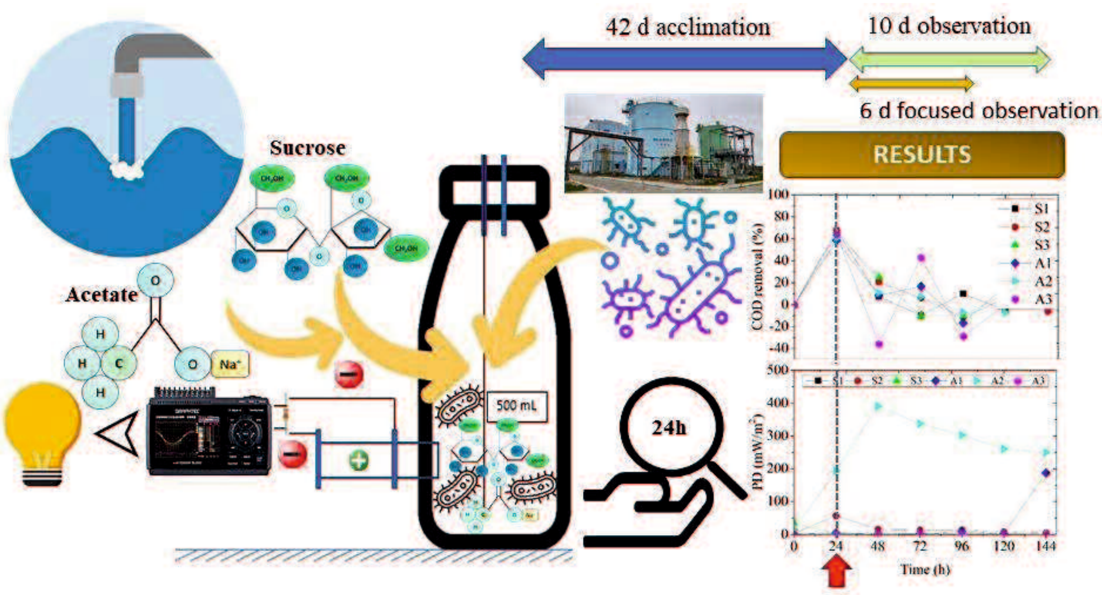

Figure 2.28 The concept of determination of optimum retention time represented of this study using AC-SCMFC

2.6. Integrated MFCs for Wastewater Treatment (MFC-WWT)

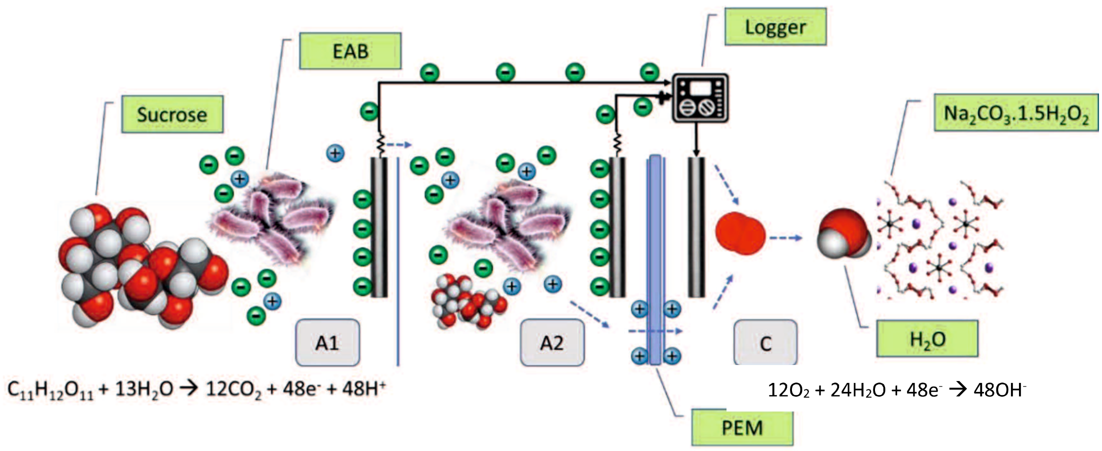

Figure 2.29 The basic design concept of the current experiment in DAC-DCMFC

One of the interesting schemes is treating the wastewater containing a high organic loading in Anaerobic Digester (AD); subsequently, a low organic loading output of AD is treated by a stack MFC system or bioelectrochemical-assisted microbial reactor (BEAMR) to produce a very low organic loading complying the water effluent standard and high-power production at the end of treatment (Aelterman et al. 2006; Clauwaert et al. 2008). Hence, this study is structured more closely with the integrated MFC-WWT in which the first anode chamber has the same function as wastewater treatment and the second anode chamber has the function of transferring abundantly generated electrons from the first anode chamber, although the process is similar to the first anode chamber. In detail, the complete explanation is shown in Chapter IV about Enhancement of Power Generation and Organic Removal in Double Anode Chamber Designed Dual-Chamber Microbial Fuel Cell (DAC-DCMFC), which is published in WATER MDPI journal (https://www.mdpi.com/2073-4441/13/21/2941).

G. Samudro, Imai, and Hung (2021) briefly figured out the conceptualization in **Figure 2.29** and **Figure 2.30**.

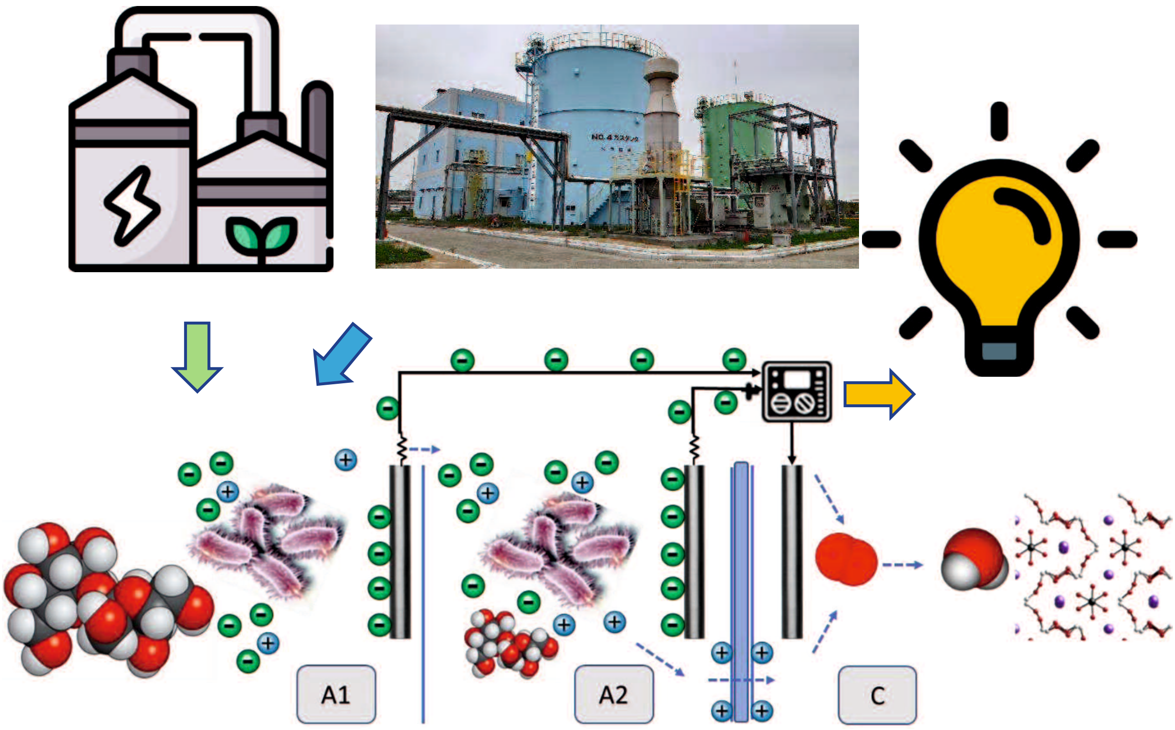

Figure 2.30 The concept of wastewater treatment integrated MFCs represented of this study using DAC-DCMFC

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CHAPTER III

DETERMINATION OF OPTIMUM RETENTION TIME IN AN AIR-CATHODE SINGLE-CHAMBER MICROBIAL FUEL CELL BATCH-MODE REACTOR BY COMPARING DIFFERENT SUBSTRATE TYPES AND CONCENTRATIONS

3.1 Introduction

With a growing population, energy demand gradually increases, suppressing an increase in other related sectors such as wastewater generation, which is a significant problem in developing countries. Currently, conventional wastewater treatment plants consume large amounts of energy, particularly electricity (Capodaglio and Olsson, 2020; Ranieri, Giuliano and Ranieri, 2021). Wastewater contains various carbon and nutrients that can be utilized as energy generation sources (Zhang *et al.*, 2019; Mohsin *et al.*, 2021). Many countries compete for renewable energy resources, but there are several constraints on developing them on a practical level. Findings show that they are more environmentally friendly in terms of reaching environmental sustainability targets, particularly with regards to energy-development investments in collaboration with private partners (Bekun, 2022). However, there is a lack of knowledge and practice with regards to their integration for enhancing wastewater engineering processes based on bioenergy technology converters, while simultaneously revaluing the final product dealing with national and international regulations, standards, and guidelines to promote the benefit of renewable energy sourced wastewater.

Nowadays, energy converters using biomass, which is contained in biological wastewater, are classified into three broad types of processes: direct combustion, thermochemical, and biochemical processes (Agrela *et al.*, 2019). Biochemical processes have become the primary method for semi-solid and liquid biomass conversion for fermentation products, biogas, fertilizers, electricity, and pure water. This study used a technique known as the bioelectrochemistry process, which directly produces electricity and simultaneously reduces organic pollutants in wastewater. Microbial fuel cells (MFCs), a major biochemical technology that converts organic matter to direct bioelectricity, have been extensively studied since 1911 by Potter and resumed by Cohen in 1931 (Flimban *et al.*, 2019). Presently, MFCs have been developed for integration with wastewater treatment systems to provide high efficiency in decontamination and electricity production (Capodaglio, Bolognesi and Cecconet, 2021). The performance of MFCs is approaching the realization of their application in the field, but limitations still exist, one of which is the RT which affects the design and operation of the reactor. A lack of information regarding the standard RT criteria applied in the MFCs is another major limitation. In addition, the problem of the impracticality of applied RT in many types of

MFC research should be comprehensively discovered to ensure high performance of MFCs in both single-chamber (SCMFC) and dual-chamber (DCMFC). It is important to carefully determine the initial substrate concentration, taking all factors into account; thereafter, the MFC system can be operated at optimal efficiency (Gadkari, Shemfe and Sadhukhan, 2019). A shorter RT lowers organic and inorganic removal even though it provides a higher power output (Ye *et al.*, 2020; Sumisha and Haribabu, 2021); a longer RT may result in lower current and power density (PD) even when dealing with organic and inorganic removal (Chang *et al.*, 2018). Additionally, an optimum RT is always found owing to linear results with organic and inorganic removals, but they are inverse with the current density for power generation (Malekmohammadi and Mirbagheri, 2021). We found that an optimal RT built on the high performance of organic removal and power production by MFCs is still a drawback even when applied under similar conditions. Therefore, the optimal RT must be determined to enable practical MFC research.

The RT is correlated to the organic loading rate (OLR) and flow rate (Q) (Nawaz *et al.*, 2020). The reactor design of MFCs for fed-batch or continuous mode, particularly on the anode chamber, can be obtained from their volumes by multiplying Q by either RT or the influent concentration (So) divided by OLR. In the case of the RT, it could be obtained from the relationship between concentration (C) and detention time (t) as with the RT in batch mode using pseudo-first-order kinetics from the following equation ($\ln C/Co = -kt$). Therefore, the optimum RT range can be efficiently determined through the batch mode. Another term for RT is hydraulic retention time (HRT), which correlates to OLR. Conventionally, HRT is mainly used in fed-batch and continuous modes owing to Q, So, and OLR correlations. A higher HRT leads to a lower OLR, which affects the operational flow of the process (Labatut and Pronto, 2018; Jung and Pandit, 2019). HRT added to Co and reactor volume (V) is highly correlated with power generation (Samudro *et al.*, 2021; Yu, 2021). Similarly, the HRT applied in many types of MFC research substantially affects organic and inorganic removal, bacterial communities, energy recovery, dissolved oxygen (DO), biofouling, and capital expenditure associated with bioreactor size, as reported elsewhere. Hence, physical, chemical, and biological processes are strongly influenced by HRT. Generally, the RT in various types of MFC research can be divided into two types: less than or equal to 24 h and more than 24 h. Retention time less than or equal to 24 h is mostly utilized in both SCMFC and DCMFC, whereas a retention time of more than 24 h is mainly applied in DCMFC as opposed to SCMFC. However, various RT determinations were undefinable. It is difficult to conclude the optimum RT range for different experiments, even under the same conditions.

Substrates are the carbon, nutrient and energy sources, and they widely used in MFC research are divided into two categories based on substrate type: single and complex substrates (Fadzli, Bhawani and Rania, 2021). Substrate concentrations are mainly defined as low (250–750 mgCOD/L), medium (>750–2250 mgCOD/L), and high (>2250 mg COD/L) strength levels (COD: chemical oxygen demand) (Stoll, Dolfing and Xu, 2018). The single substrate, mostly sugar as a carbon source, is known to achieve high performance; meanwhile, a complex substrate, mainly natural wastewater, is closely related to realization. In addition, either concentration or loading of substrates often divers in many types of MFCs operation, exceptionally high concentrations stimulate high power output but low organic removal, and conversely. Because substrate type and concentration have a significant influence on the current generation of MFCs (Yu, 2021), they are the main variables that should be addressed for a better understanding of the optimum RT.

A new approach employed in the batch process in MFC studies could make it easier to obtain the optimum RT (Thompson, Runge and Dunne, 2019; von Sperling, Verbyla and Oliveira, 2020). Here, the optimum RT related to oxidizable or biodegradable substances could be assigned as design criteria for many MFC types. Therefore, the objective of this study was to determine the optimum RT for various types of MFC, and the AC-SCMFC batch-mode reactor was tested by comparing different types and concentrations of substrates based on the primary parameters of organic removal and power generation. In order to attain the main purpose of this study, sufficient and prominent time observations were made to help understand the phenomenon caused by the regressors and to determine the optimum RT. The comprehensive retention time analysis was carried out in a shorter time, specifically over 6 d, which revealed a significant pattern and has the potential to serve as design criteria. Two of the most widely used substrates in various types of MFC studies and their three concentration levels were employed to determine the significant pattern. Total organic carbon (TOC) was added as an organic parameter to ensure the correct measurement of COD and to further explain the phenomenon of change or removal of organic matter contained in wastewater.

3.2 Materials and Methods

3.2.1 AC-SCMFC configuration

A schematic configuration of the AC-SCMFC reactor is shown in **Figure 3.1**. The reactor was arranged with several supporting components: a polypropylene plastic (PP) bottle chamber (volume 650 mL), an anode, a cathode, and a resistor-equipped wiring system connected to a 24 h data logger.

The working volume was set at 500 mL. The electrodes were prepared using graphene nanoplatelets (GNPs) purchased from STREM Chemicals, Inc. (Newburyport, MA 01950 USA). The anode was set up by heating a stainless steel mesh (SSM) that had previously been immersed in a GNPs solution (1 g/L) in four cycles. The cathode was prepared by soaking the SSM in the GNPs:PU solution (1:1) and air-drying it for 24 h for a desirable waterproofing effect. GNP-based electrodes were used to enhance electron capture on the surface area of anode-based nanoparticles and simplify the transfer to cathode-based nanoparticles, thereby providing a high potential between electrodes because of their special characteristics, particularly in terms of electrical conduction. The external resistor applied at 100 Ω was based on many references, particularly on SCMFC research. This was connected to a data logger using midiLOGGER GL-240 (GRAPHTEC, Graphtec Corporation, Japan) with a 30 min sampling time.

Figure 3.1 Schematic illustration of AC-SCMFC

3.2.2 Enrichment and operation of AC-SCMFC

The electrochemically active bacteria (EAB) enrichment in the AC-SCMFC was performed in respirometry-batch mode. The potential EAB species was performed by polymerase chain reaction (PCR) that discovers genetic material and denaturing gradient gel electrophoresis (DGGE) that expresses bacteria communities. 27 F and 1492 R were employed as universal 16S rRNA gene primer based PCR products from DNA extraction, and GC-341 F and 518 R were utilized as universal 16S rRNA gene primer based DGGE. Additionally, an EAB community analysis, which plays an essential role in the energy industry, followed the hazard assessment for biomass gasification stations using a general set-pair analysis (Yan *et al.*, 2016) and novel extended set-pair analysis (Yan *et al.*, 2017). Anaerobic sludge was obtained from the Eastern-Ube sewage treatment plant (EU-STP) located in Ube City, Yamaguchi Prefecture, Japan. The inoculation used for seeding was injected at approximately 30% of the 500 mL working volume of the respirometry-bottle chamber during the observation period. The synthetic wastewater for feeding, which served as carbon and nutrient source, consisted of 5.93 g/L of sucrose, 4 g/L of $NaHCO_3$, 4 g of K_2HPO_4, 2 mL/L of trace element A ($(NH_4)_2HPO_4$ 350 g/L), 10 mL/L of trace element B (KCl 75 g/L, $MgCl_2.6H_2O$ 81 g/L, NH_4Cl 85 g/L, $MgSO_4.7H_2O$ 25 g/L, $FeCl_3.6H_2O$ 42 g/L, $CoCl_2.6H_2O$ 1.8 g/L), and 1 mL/L of trace element C ($CaCl_2.6H_2O$ 150 g/L) (Zhou *et al.*, 2006, 2007). The other substrate was acetate-based synthetic wastewater and consisted of 6.24 g/L of sodium acetate with the same nutrient and trace element components as those in the sucrose-based substrate. All pro-analysis (p.a) high-quality carbon, nutrient, and trace element components were obtained from Wako Pure Chemical Industries, Ltd., Japan. In addition, two types of saccharides consisting of monosaccharides (acetate), which are represented as highly oxidized, and disaccharides (sucrose), and are defined as oxidizable, are utilized because they are extensively used in various types of MFC studies and can be represented as part of real wastewater that can be treated properly.

The basis for using COD levels was based on a literature review, preliminary experiment using a respirometric chamber analysis, and the preliminary observation of cell voltage patterns among five different COD levels, which were 400, 1000, 2500; 5000; and 10,000 mg/L. Stoll et al. (2018) employed COD inputs of 250, 750; 2250 mg/L as the assumed COD represents the actual wastewater strength levels as low, medium, and high, respectively, to achieve energy-neutral wastewater treatment of the minimum performance requirements in the MFC. As a result, respirometric chamber analysis concluded that a COD level of more than 2500 mg/L indicated minimal COD removal. Similarly, the cell voltage pattern was insignificant with a COD level of more than 2,500 mg/L. As noted, respirometric chamber analysis and cell voltage pattern results used as preliminary observations have not been published elsewhere.

The AC-SCMFC batch mode was operated under anaerobic conditions by purging the synthetic wastewater with pure nitrogen gas (N_2), indicating null oxygen interference. This process occurred in parallel with different carbon sources for 52 d. Before being injected with new substrates for recording observations, the reactors were acclimated within 42 d to ensure the stabilization of the process, including organic removal and power generation. After the acclimation period, the reactors were activated for 10 d for the main observation, during which the physical and chemical parameters were assessed and recorded every 24 h.

3.2.3 Physical and chemical analysis

Physical analyses were required to ensure and control the chemical processes. Measurements of pH and temperature and ORP were performed using a pH meter D-72 (HORIBA Advanced Techno Co., Ltd., Kyoto, Japan) and a pH/ORP meter D-72 (HORIBA Advanced Techno Co., Ltd., Kyoto, Japan), respectively. The chemical analyses included optical density measurement at 600 nm (OD_{600}), COD analysis, and estimation of TOC. The OD_{600} was analyzed using a spectrophotometer (U-2900, Hitachi

High-Tech Science Corporation, Tokyo, Japan). COD and TOC were assessed according to the standard methods (APHA, AWWA and WEF, 2017).

The percentage of COD and TOC removal, symbolized as [C] removal, was calculated as Eq. (1).

$$[C]\ removal = \frac{[C]_{in} - [C]_{out}}{[C]_{in}}\ x\ 100\% \quad (1)$$

where $[C]_{in}$ (mg/L) is the COD and TOC of the influent, and $[C]_{out}$ (mg/L) is the COD and TOC of the effluent.
The kinetic rate of COD changes was enumerated through pseudo-first-order kinetics that can be expressed as Eq. (2).

$$\frac{Ct}{Co} = e^{-kt} \quad (2)$$

where Ct (mg/L) is the COD of the effluent, Co (mg/L) is the COD of the influent, k (/d) is the kinetics rate or reaction rate, and t (d) is the retention time.

3.2.4 Power generation analysis

The power in the voltage unit was recorded using a data logger with a specification at the AC-SCMFC configuration, whereas the current in amperes was calculated by following Ohm's law as Eq. (3).

$$I = \frac{V}{R} \quad (3)$$

where I is current (A), V is the cell voltage (V), and R is the resistance (Ω).
The power density (PD) was enumerated as Eq. (4).

$$PD = V \times \frac{I}{A} \quad (4)$$

where PD is the power density (W/m^2), V is the cell voltage (V), and A is the surface area of the anode carbon (m^2) (Hejazi, Ghoreyshi and Rahimnejad, 2019).
Coulombic efficiency (CE) was computed Eq. (5).

$$CE = \frac{MC}{Fz.v.\Delta COD}\ x\ 100\% \quad (5)$$

where CE = coulombic efficiency (%), F = Faraday's constant (96,485 C/mol), M = molecular weight of oxygen (32 g/mol), z = 4 (the number of electrons exchanged per mole of oxygen), ΔCOD = difference in the influent and effluent COD (g/L), Cs = charge passed (A s), and v = anodic volume (L) (Logan *et al.*, 2006).
Internal resistance was measured by varying the external resistance from 10 kΩ to 1 Ω using a resistance box and calculated based on the V-I relationship of the linear portion of the polarization curve (Watson and Logan, 2010). The internal resistance equation was calculated Eq. (6).

$$R_{int} = \frac{\Delta V}{\Delta I} \quad (6)$$

where R_{int} is internal resistance (Ω), ΔV is the cell voltage difference (V), and ΔI is the current difference (A).

3.2.5 Data acquisition

The analysis data were collected by screening it based on the performance of the AC-SCMFC from the beginning of the running time, which was useful for mapping the pattern of both the main parameters in organic removal and power generation. Likewise, the present data on seeding and feeding as initial and control data were properly recorded to ensure precise results during time observation. Prior to selecting a significant pattern to simplify the determination, which focused on a shorter time, the overall analysis based on power generation, which was cell voltage, and organic removal, which was COD and TOC removals, was revealed to provide a better understanding of the argument. The optimum RT was investigated by comparing substantial factors including COD removal (CODr), the reaction rate of COD (kCOD), TOC removal (TOCr), and the reaction rate of TOC (kTOC), which represents organic removal, and cell voltage, PD, and CE, which represent power generation. CODr and TOCr are the pinpoints for rapidly revealing organic removal-based carbon sources. The CODr and TOCr equations can be obtained using Eq. (1). Meanwhile, kCOD and kTOC provided additional support for COD removal to show the precise retention time for converting organic substrates to produce electrons, protons, and several products. The kCOD and kTOC equations can be obtained using Eqs. (2). Furthermore, the cell voltage is essential for calculating the PD and CE. It is necessary to determine the actual power generation in addition to the cell voltage. The PD and CE equations can be obtained using Eq. (4) and Eq. (5), respectively. Moreover, additional supporting data is the internal resistance, which can be used to evaluate the performance of ohmic resistance by anode, cathode, and substrate effects. The internal resistance equation can be obtained using Eq. (6). Different substrate types and concentrations that affect the optimum RT findings are worthwhile when designing any MFC reactor type related to integrated MFC compliance for wastewater treatment, notably for anaerobic or anoxic biological treatment. The optimum RT is defined as the detention time (t) obtained in this study, which follows Eq. (2). Furthermore, t can be used to enumerate the volume of the MFC reactor in fed-batch or continuous mode. For further studies, the volume was expressed by Eq. (7).

$$v = Q\ x\ t \tag{7}$$

where v is the anodic volume (L or m^3), Q is the flow rate (L/d or m^3/d), and t is detention time (d). Moreover, the known volume can be used to compute the surface area of the anodic chamber by first assuming the height of the chamber and vice versa. In correlation with the OLR, t as the HRT can be obtained by Eq. (8).

$$t\ (HRT) = \frac{Q\ x\ Co}{Q\ x\ OLR} = \frac{Co}{OLR} \tag{8}$$

where Q is the flow rate (L/d or m^3/d), Co (≈ So) is the input/initial concentration of organic matter (mg/L or g/L), and OLR is the organic loading rate (kg/m^3.d or g/L.d).

Subsequently, the correlation of the volume with the OLR was calculated by combining Eq. (7) and Eq. (8) as Eq. (9).

$$v = Q\ x\ \frac{Co}{OLR} \tag{9}$$

where v is the anodic volume (L or m^3), Q is the flow rate (L/d or m^3/d), Co (≈ So) is the input/initial concentration of organic matter (mg/L or g/L), and OLR is the organic loading rate (kg/m^3.d or g/L.d).

Furthermore, all data from different independent variables were analyzed and compared to determine the optimum RT.

3.3 Results and Discussion

3.3.1 Seeding and feeding characteristics

Seeding was used to ensure that biological processes occurred in the system. Seeding and feeding had the following characteristics (**Table 3.1** and **Table 3.2**).

Table 3.1 Seeding characteristics

pH	7.12 ± 0.04
Temperature	20.3 ± 6.0 °C
COD	396 ± 3 mg/L
BOD_5	198 ± 1 mg/L
$P\text{-}PO_4$	266 ± 4 mg/L
$N\text{-}NO_3$	26 ± 3 mg/L
Total solids (TS)	12,740 ± 144 mg/L
Total suspended solids (TSS)	11,973 ± 693 mg/L
Volatile suspended solids (VSS)	8415 ± 423 mg/L
Fixed suspended solids (FSS)	3320 ± 178 mg/L
EAB species	*Arcobacter butzleri, Arcobacter lekhitochrous, Lactococcus laudensis, Schwartzia succinivorans, Selenomonax lacticifex, Clostridium polynesience, Clostridium aurantibutyricum*

The characteristics of seeding showed appropriate initial conditions for the potential growth of EAB and its adaptation in AC-SCMFC reactors; hence, seeding can be directly inoculated into the system without any pretreatment or conditioning. The pH was neutral, whereas the temperature range was small and at the ambient level. A neutral pH during seeding can optimize the performance of MFCs (Rozendal, Hamelers and Buisman, 2006; Puig *et al.*, 2010). Similar studies stated that a pH between 6 and 9 in various anolyte types improved the performance of any type of MFC (Kumar and Mungray, 2016; Bagchi and Behera, 2021). E-proteobacteria, firmicutes, and various observed microorganisms can live appropriately at temperatures between 0–45 °C in which 0–20 °C is best for psychrophilic bacteria (Gounot, 1986; Sandle and Skinner, 2013), and 20–45 °C for mesophilic bacteria (Cavicchioli, 2016). EAB species can be classified as pathogenic bacteria (Lerma *et al.*, 2014; Sun *et al.*, 2016); however, they have the advantage of producing and transferring electrons simultaneously (Fedorovich *et al.*, 2009; Kracke, Vassilev and Kromer, 2015; Moscoviz *et al.*, 2017). COD, P-PO_4, N-NO_3, TS, TSS, VSS, and FSS are used to denote the concentration levels. COD concentrations lower than 1000 mg/L were used as benchmarks for further observation. However, the other parameters followed linearly with COD concentration.

Table 3.2 Feeding characteristics

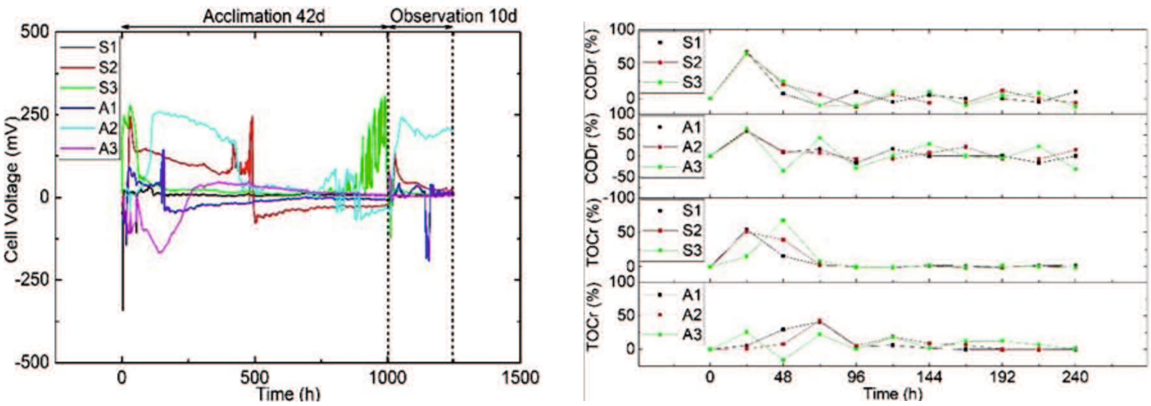
Parameters	Sucrose			Acetate		
	S1	**S2**	**S3**	**A1**	**A2**	**A3**
pH	7.47 ± 0.09	7.82 ± 0.06	7.89 ± 0.06	7.77 ± 0.1	8.24 ± 0.18	8.49 ± 0.06
Temperature (°C)	20.3 ± 0.25	19.7 ± 0.15	19.1 ± 0.12	19.1 ± 0.12	19.4 ± 0.1	18.8 ± 0.1
ORP (mV)	-22 ± 2	-16 ± 3	-26 ± 2	-23 ± 1	-21 ± 1	-40 ± 1
OD_{600} (Abs)	0.571 ± 0.04	0.059 ± 0.01	0.140 ± 0.02	0.022 ± 0.001	0.072 ± 0.003	0.306 ± 0.01
COD (mg/L)	480 ± 35	1,160 ± 153	3,000 ± 764	450 ± 21	920 ± 171	2,600 ± 153
TOC (mg/L)	77 ± 12	245 ± 49	590 ± 86	73 ± 6	171 ± 12	494 ± 72

Note: S1, S2, and S3 represent low, medium, and high concentrations of sucrose, respectively; A1, A2, and A3 represent low, medium, and high concentrations of acetate, respectively.

As shown in **Table 3.2**, the pH in acetate was higher than that in sucrose. A pH input of feeding between 7–8 could escalate the performance of MFCs (Zhang *et al.*, 2011; Bagchi and Behera, 2021). Meanwhile, the temperature showed a slight difference between sucrose and acetate. The temperature was similar to the pH mentioned above; nevertheless, it was acceptable for the system. The ORP substrates showed negative results, meaning that the initial treatment purged by N_2 was accepted correctly to ensure the next step. The OD_{600} in acetate was higher than that in sucrose. OD_{600} estimates the concentration of bacterial cells through the visibility of sample density in a spectrophotometer at a wavelength of 600 nm (Shao *et al.*, 2016; Stevenson *et al.*, 2016). COD and TOC concentrations of sucrose were higher than those of acetate. The initial concentrations of COD and TOC were correctly injected into the system. Similar to seeding, feeding can be delivered directly to the system.

3.3.2 Overall performance

The overall performance-based cell voltage and organic removal were conducted for RT range selection. For example, **Figure 3.2** shows that the acclimation time over 42 d is shown by positive cell voltage from 0 h to 500 h, after which it declines to negative cell voltage from 500 h to 1000 h. Therefore, based on the acclimation time observation, the focused observation was understood to be 240 h or 10 d. For further analysis, the observation period was then shortened to 144 h or 6 d because of the performance significance of cell voltage and organic removal, and it was, therefore, easier to conclude. Moreover, it can be seen that after an RT of 144 h or 6 d, the performance was stable, and there were no substantial changes. However, the maximum RT period was limited to 240 h or 10 d to avoid overestimation.



(a) (b)

Figure 3.2 Overall performance of AC-SCMFC in various substrate types and concentrations; (a) cell voltage-based performance, (b) organic removal-based performance

In addition, the overall substrate types and concentrations showed that acetate was higher in cell voltage than sucrose, whereas sucrose was higher in organic removal than acetate. The sucrose-based substrate was utilized faster than the acetate-based substrate because the Gibbs free energy of sucrose was more negative (approximately -1320.10 kJ/mol) (Joback and Reid, 1987) than the Gibbs free energy of acetate (approximately -607.2 kJ/mol) (Haynes, 2017). A lower Gibbs free energy indicates more spontaneous oxidation by the oxidizing agent without added energy inside the system (Papamichael *et al.*, 2019). This phenomenon could be represented at the same time of 72 h, in which the COD and TOC removal accumulation of the sucrose-based substrate was more than 90%, while the acetate-based substrate attained less than 80%. Another related reference using different monosaccharides revealed faster utilization of 12 different monosaccharides by the acclimated mixed cultures of *Rhodococcus* sp. and *Paracoccus* sp. with a COD removal of up to 80% compared to acetate resulting in a removal of 59% at the same time (Catal *et al.*, 2008). COD removal depends on the reactor operation conditions, microbial communities, and a large electrode surface area (Zhang *et al.*, 2015). Thus, the acetate-based substrate as a carbon source has a long-period advantage in the MFC system; nevertheless, the sucrose-based substrate has a short-period advantage and enhances earlier power generation. This finding contributes to several types of MFC research on single substrate utilization to achieve higher performance and faster use of substrates; accordingly, the selection of substrate types can be considered from the Gibbs free energy, which influences the performance of both organic removal and power generation, in addition to considering other related-significant effect factors. As for complex substrates, these single substrates can be classified into oxidizable/biodegradable substances or non-oxidizable/non-biodegradable substances based on the TOC/COD or BOD/COD ratios to uniquely identify the range of complex substrates. Interestingly, this ratio represents greater recognition and acceptance of further wastewater treatment.

3.3.3 Control parameters

Figure 3.3 shows that pH, temperature, ORP, and OD_{600} were in the acceptable range of the MFC experiments. A pH of 6–8 is required for all types of MFC research (Liu *et al.*, 2010; Puig *et al.*, 2010), as well as a mesophilic temperature of 20–45 °C (Goto and Yoshida, 2019; Kloch and Toczylowska-Maminska, 2020), an ORP of -200 to -400 mV (Watson and Logan, 2010; Flimban *et al.*, 2019), and an OD_{600} of 0–1 Abs (Fakhirruddin *et al.*, 2018; Nguyen *et al.*, 2020). The acetate pH was higher than the sucrose pH, whereas the pH at low strength substrate of sucrose and acetate was higher than with the medium and high-strength substrates. The temperature of sucrose and acetate was consistently around 20 °C over the observed time. As with the pH, the ORP acetate was higher than the ORP sucrose, while the low strength acetate substrate was higher than the medium and high strengths. Nevertheless, the ORP at the low strength sucrose substrate was lower than the medium and high strengths. The OD_{600} of sucrose was higher than acetate. Interestingly, sucrose's high-strength synthetic wastewater gradually increased from 0 to 48 h and was higher than the low- and medium-strength substrates. Whereas the high-strength acetate substrate showed a similar result to sucrose, the ORP was constantly decreasing to reach -400 mV between 0 to 48 h. It was reported that the ORP did not vary compared to the power output (Watson and Logan, 2010). The above-mentioned fourth parameter pattern was not significant when determining the RT as the patterns did not exhibit the optimum position at a specified time. Likewise, G. Samudro et al. (2021)

observed the parameters of pH, temperature, ORP, and OD_{600} in DAC-DCMFC, which did not show a significant difference during the 192-hour running period. In brief, for this condition, the control parameters worked in controlling the process.

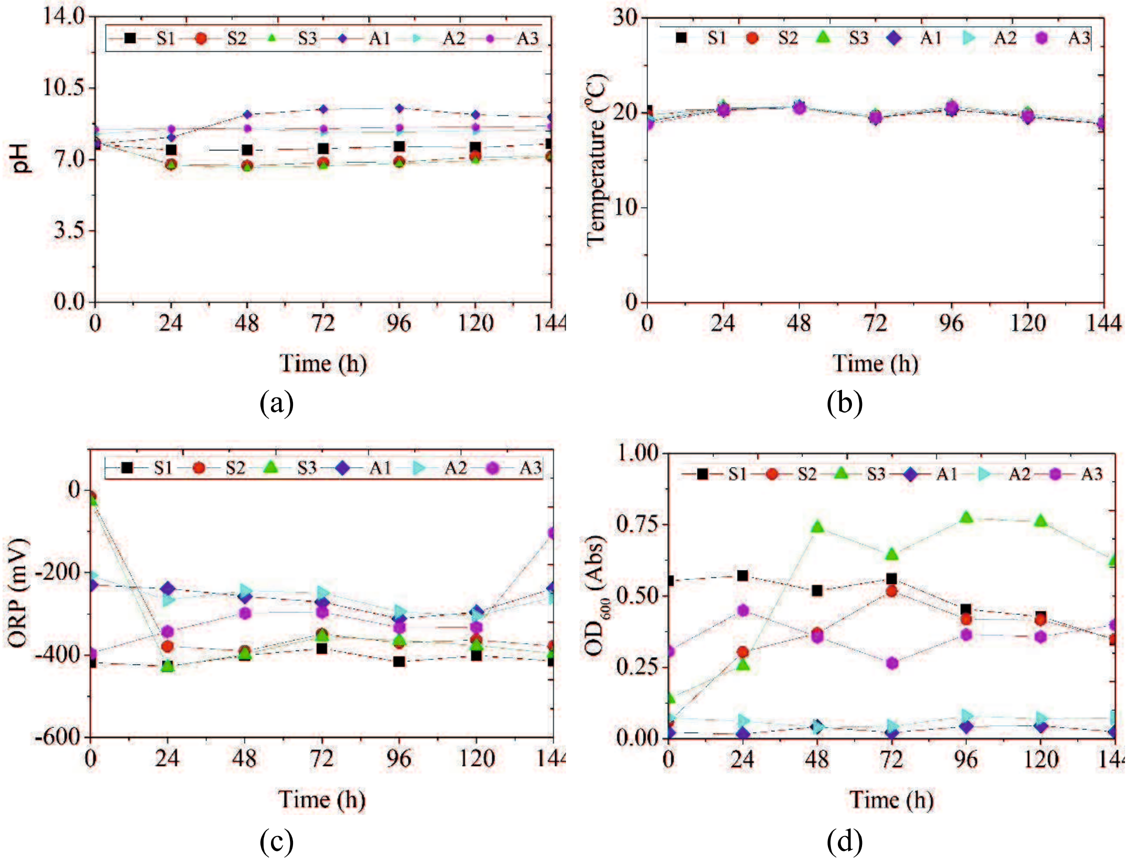


Figure 3.3 Comparison of control parameters affected by different substrate types and concentrations; (a) pH, (b) Temperature (ºC), (c) ORP (mV), (d) OD_{600} (Abs); S1, S2, and S3 = low, medium, and high concentrations of sucrose, respectively; A1, A2, and A3 = low, medium, and high concentrations of acetate, respectively

3.3.4 Organic removal

Figure 3.4 shows the organic changes, removal, and kinetic rate of both substrates and three concentrations with COD and TOC as the organic parameters. In detail, the correlation between COD changes, removal, and the kinetic rate of both substrates and the three concentrations reached an optimum level at 24 h. However, the TOC removal and kinetic rate mainly occurred at the high-strength level of both substrates and achieved the optimum at 48 h. This happened because a high-strength level contains a high level of total carbon, which is used by the EAB, compared to the low- and medium-strength levels. Interestingly, the COD and TOC performances showed peak results and were stable at 24 h rather than 48 h onwards, described as unstable, especially at high-strength levels of both substrates. Therefore, when considering this for practical use, the recommended RT could be of concern at 24 h, thus affecting the design of the MFC reactor.

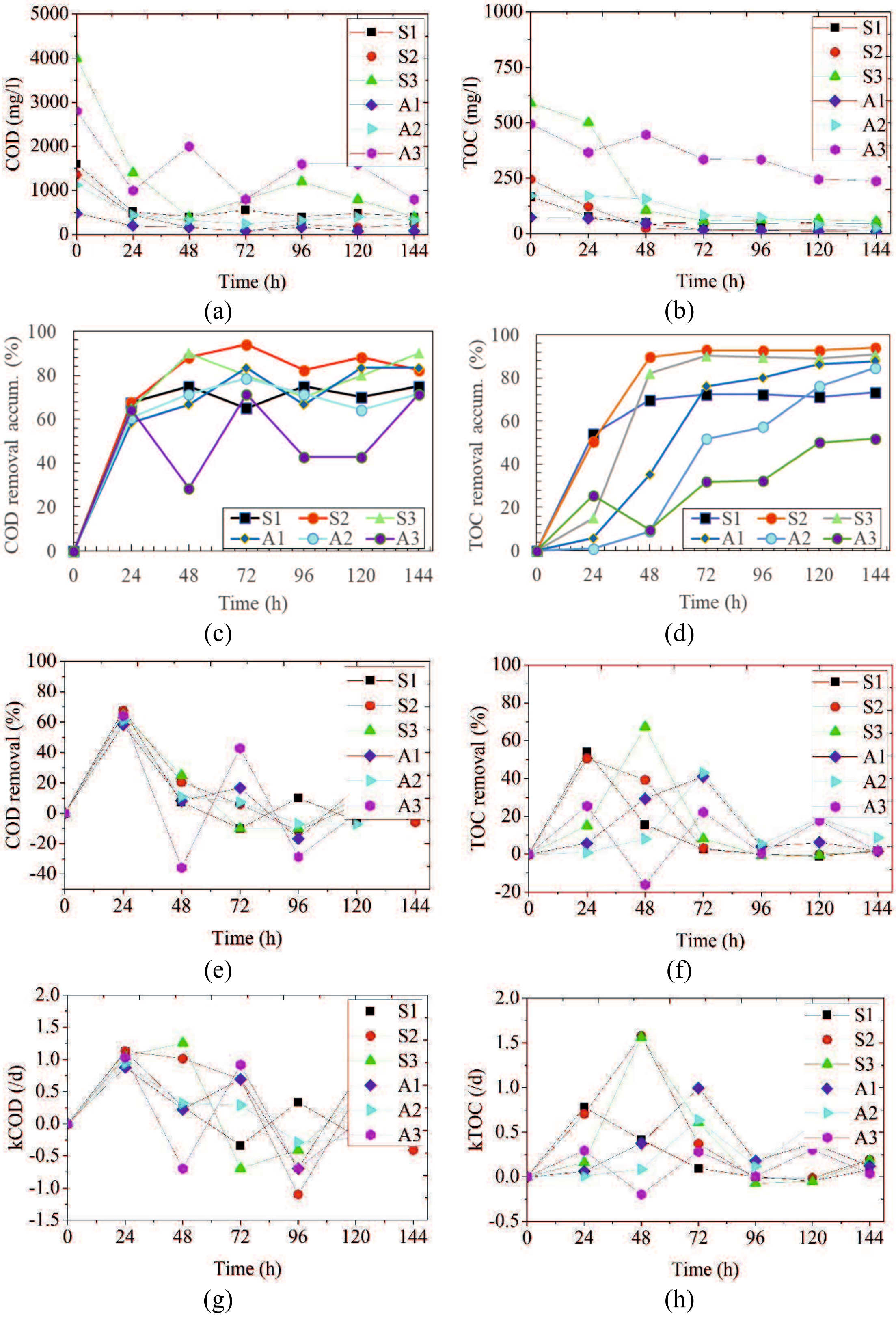


Figure 3.4 Comparison of organic removal affected by different substrate types and concentrations; (a) COD changes, (b) TOC changes, (e) COD removal accumulation (%), (f)

TOC removal accumulation (%), (e) COD removal (%), (f) TOC removal (%), (g) kCOD (/d), (h) kTOC (/d)

At 24 h, COD and TOC removal of both substrates and the three concentrations reached the ranges of 50–70% and 0–60%, respectively. COD removal in various studies ranges from 36–84% (Linares *et al.*, 2019). Meanwhile, TOC removal in several studies with the same concern achieved ranges from 60–95%. Therefore, both COD and TOC removal can be stated on the typical performance of MFC research. Thus, the AC-SCMFC batch mode is acceptable for achieving the minimum performance requirements for MFC studies to obtain significant patterns. Regarding the optimum COD and TOC removal, the medium-strength level of sucrose reached a higher COD removal than others, while sucrose's low- and medium-strength levels showed a higher TOC removal than the others. Therefore, low and medium strength levels could be established for COD and TOC removal.

First-order kinetics is an approach to help understand the COD removal rate, which depends on the operating conditions present (Zhang *et al.*, 2015). The COD and TOC kinetic rates of both substrates and the three concentrations were in the ranges 0.75–1.25/d and 0–1.0/d, respectively. Based on the first-order kinetics approach, the RT substantially affects COD removal with an average kinetic rate of 1/d; meanwhile, a longer RT is needed for TOC removal with an average kinetic rate of 0.5/d. A similar configuration to this study, based on a dynamic computational model, represents the rate of bioanode kinetics on a glucose-based substrate of 0.84/d (Gadkari, Shemfe and Sadhukhan, 2019). As with COD and TOC removal, the COD and TOC kinetic rates for sucrose were higher than acetate. Regarding the optimum achievement of kinetic rate, the medium-strength level of sucrose aimed to achieve the COD kinetic rate, whereas low- and medium-strength levels were related to the TOC kinetic rate. As with COD and TOC removal, the low- and medium-strength levels could be adjusted to the COD and TOC kinetic rates.

Because COD and TOC kinetics have the same inference as COD and TOC removal, one of these indicator parameters can be chosen to determine the performance of each MFC study. Interestingly, the kinetic rate provides additional quantitative information on the process that describes the change in organic substances through biochemical processes during a specified time in the MFC reactor. Therefore, further studies should consider the kinetic rate used to monitor the results of COD and TOC removal at fixed or determined RT.

3.3.5 Power generation

Figure 3.5 shows the power generation performance for different substrate types and concentrations based on the indicators of cell voltage, surface PD, volumetric PD, and CE. The relationship between the cell voltage, surface PD, volumetric PD, and CE with a medium concentration of sucrose definitively achieved the optimum RT at 24 h. Nevertheless, the fourth indicator displayed a significant pattern with a medium concentration of acetate and reached the optimum RT at 48 h. The low and high substrate levels did not exhibit a significant pattern; therefore, they were not used to determine the optimum retention time. Organic removal was significantly related to power generation due to the similarity, especially the performance indicator at 24 h. We found that acetate performed substantially in the power generation indicators, particularly at 48 h. Therefore, the recommended time for both substrates and the three concentrations was set at 24 h. This resulted in a change in the power generation indicator even though the acetate still shifted to the peak at 48 h.

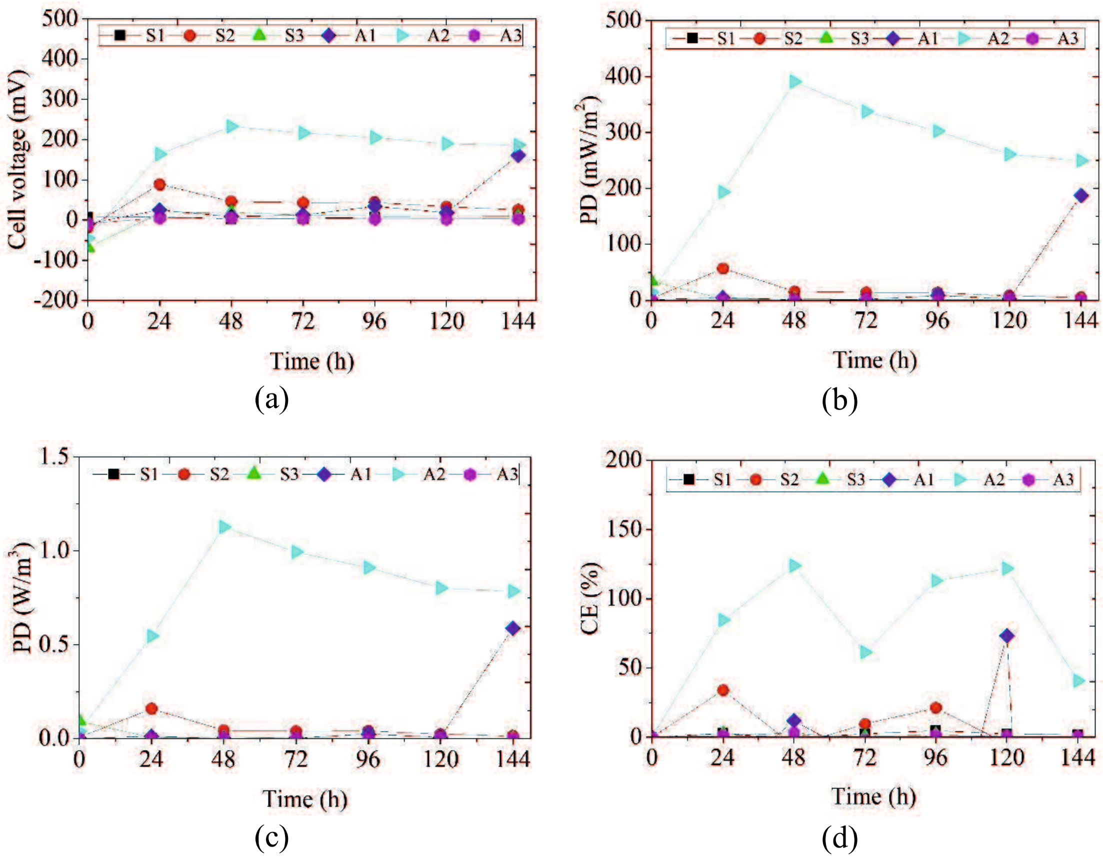
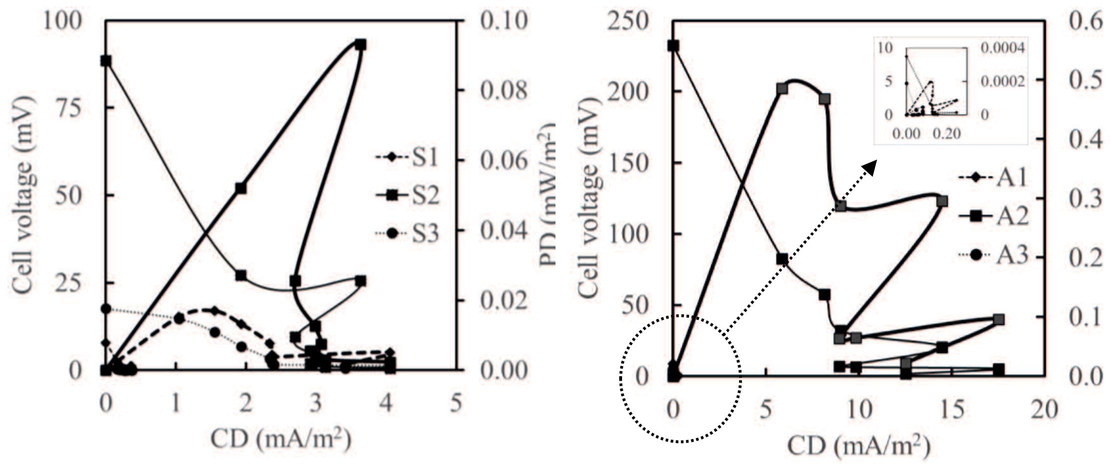


Figure 3.5 Comparison of power generation affected by different substrate types and concentrations; (a) Cell voltage (mV), (b) Surface power density (mW/m^2), (c) Volumetric power density (W/m^3), (d) Coulombic efficiency (%)

At 24 h, power generation of both substrates and the three concentrations attained cell voltage in the range of 50–100 mV at a medium sucrose level, 150–200 mV at a medium acetate level, and 0–50 mV at low and high levels of both substrates. Sleutels et al. (2016) stated that a cell voltage of more than 100 mV ensures that EAB is more dominant than methanogenic bacteria. In this study, the acetate satisfied the cell voltage performance, especially at the medium concentration level, which means that the EAB achieves the point when the substrate concentration is medium. Meanwhile, sucrose did not reach more than 100 mV, which means that its performance did not increase the cell voltage. This was similar to the conditions of the low and high concentrations of the two substrates, which were not suitable for obtaining cell voltages. In addition, a surface PD was achieved in the range 50–100 mW/m^2 at a medium sucrose level, 150–200 mW/m^2 at a medium acetate level, and 0–50 mW/m^2 at low and high levels of both substrates. Meanwhile, volumetric PD reached the range 0–0.25 W/m^3 at medium sucrose level, 0.5–0.75 W/m^3 at medium acetate level, and 0–0.25 W/m^3 at low and high levels of both substrates. As with a previous study that focused on the applied optimum RT in AC-SMCFC, the maximum PD was achieved at 1155 ± 41 mW/m^2 when the cathode side was assisted by the membrane to minimize oxygen penetration and subtract biofilm formation (Vogl, Bischof and Wichern, 2016). In contrast, the bioanodes of air-cathode microbial fuel cells (ACMFCs) reached a maximum PD of 73 mW/m^2 in recovering the mixed Sn(II), Fe(II), and Cu(II) with the co-present organics in the simultaneous treatment of printed circuit board (PrCB) synthetic wastewater (Song *et al.*, 2019). In this study, RT at 24 h provided a higher PD. Furthermore, the CE was extended to the range 25–50% at a medium sucrose concentration, 75–100 % at a medium acetate concentration, and 0–25% at low and high concentrations of both substrates. In comparison with other studies using acetate as a substrate, CE achievement was up to 70% (Rozendal *et al.*, 2007; Sleutels *et al.*, 2011). Vogl et al., 2016 reported that the membrane-equipped AC-SCMFC with a maximum PD of more than 1000 mW/m^2 achieved 54% of CE. The CE range of acetate is appropriate as the minimum provision of at least 80% for MFC optimization (Stoll, Dolfing and Xu, 2018). The medium acetate level performed very well based on the CE performance needed to optimize the MFC system. In contrast, sucrose did not achieve a minimum level of CE at any concentration. Consistent with the organic removal parameters mentioned above, the medium-strength levels of both substrates depicted the significant optimum correlation with power generation rather than low and high levels. These findings indicate that a medium concentration level could be used for further studies in any MFC research to stimulate the high performance of both organic removal and power generation. Compared with other studies, the first attempt at a concentration of 1,000 mg COD/L of acetate confirmed that the substrate concentration increased the PD of DCMFC (Ullah and Zeshan, 2020). Meanwhile, a related study using SCMFCs for treating dairy wastewater in the presence of 1,000 mg COD/L showed a maximum open-circuit voltage (OCV) in the range of 200–400 mV, an estimated PD of approximately 200 mW/m^2, a current density of approximately 500 mA/m^2, and a CE of 67.53% (Choudhury *et al.*, 2020). The maintained COD concentration of about 1,000 mg COD/L in an anode chamber of a mixotrophic photosynthetic microbial fuel cell (PMFC) produced the maximum PD of 8.20 (± 0.42) and 10.68 (± 0.88) mW/m^2 throughout batch 1 and 2 (Yahampath Arachchige Don and Babel, 2021), respectively. Low- and high-concentration levels could be applied after treating the initial conditions of the MFC experiment with medium levels for acclimation purposes.

Figure 3.6 shows the support power generation analysis for **Figure 3.5**, in which the surface PD could be obtained from the PD curve and calculated manually following Ohm's law. The polarization curves of both the substrates depict the internal resistance present in the system. According to the slope calculation of the polarization curve, the internal resistance of sucrose ranging from a low to high concentration level was 858 Ω, 83 Ω, and 6,268 Ω, respectively. A high concentration provided higher internal resistance than low and medium levels. It could be inferred that the internal resistance could be affected by differences in the substrate level. The decreasing external resistance can influence the COD removal efficiency (Tamta, Rani and Yadav, 2020). Low and medium levels had the same requirement of applied external resistance as the high level. In this study, the applied external resistance of 100 Ω was acceptable, even though it provided low power generation. In addition, a lower external resistance than internal resistance could improve the biofilm maturation and performance (Pasternak, Greenman and Ieropoulos, 2018) and the number of transferred electrons (Cai, Qaisar and Sun, 2020). Meanwhile, the internal resistance of acetate ranging from a low to high concentration level was 1,231 Ω, 2,022 Ω, and 2,212 Ω, respectively. Similar to the R_{int} of sucrose, low and medium levels provided lower internal resistance than high level even though R_{int} of acetate showed higher than R_{int} of sucrose. Three substrates (glucose, fructose, and sucrose) with different concentrations were used to evaluate the internal resistance performance, and it has been shown that sucrose had higher internal resistance than glucose and fructose (Jafary *et al.*, 2013). The internal resistance can be affected by the anodic substrate (Baniasadi and Vahabzadeh, 2021). This study contributes to R_{int}'s information of any MFCs that R_{int} acetate has higher internal resistance than sucrose, glucose, and fructose. Furthermore, not only low R_{int} can increase power generation but also high R_{int} with optimal initial COD concentration and substrate type. Therefore, it can be concluded that the internal resistance is influenced by the substrate type.



(a) (b)

Figure 3.6 Comparison of polarization and power density curves under closed-circuit voltage (CCV) affected by different substrate types and concentrations; (a) Polarization and power density curves (sucrose), (b) Polarization and power density curves (acetate); S1, S2, and S3 = low, medium, and high concentrations of sucrose, respectively; A1, A2, and A3 = low, medium, and high concentrations of acetate, respectively

3.3.6 Factors in comparison

To determine the retention time efficiently, a simplified factor comparison was performed, as shown in **Table 3.3**. The RTs at 24 h and 48 h were chosen because they showed a significant pattern compared to other RT values.

Table 3.3 Comparison of factors between 24 h and 48 h RT with different substrate types at a medium substrate concentration

Determiner	Parameters	Unit	RT = 24 h		RT = 48 h	
			S2	A2	S2	A2
Organic removal	CODr	%	68	61	21	11
	kCOD	/d	1.13	0.93	1.01	0.32
	TOCr	%	51	1	39	8
	kTOC	/d	0.70	0.009	1.58	0.08
Power generation	Cell Voltage	mV	88.6	164	46	233
	PD	mW/m^2	56.69	193	15.55	390
	PD	W/m^3	0.16	0.55	0.04	1.13
	CE	%	34	84	-6	124
	R_{int}	Ω	83	2,022	83	2,022

Note: S2 and A2 represent medium concentrations of sucrose and acetate, respectively.

Table 3.3 shows that the CODr, kCOD, TOCr, and CE of both substrates at a medium-strength level and RT 24 h outperformed the fourth mentioned parameters at RT 48 h. Meanwhile, the kTOC, cell voltage, and PD of both substrates at a medium-strength level and RT 48 h exceeded the fourth mentioned parameters at RT 24 h. These findings reveal that RT 24 h is better for organic removal than power generation; on the contrary, RT 48 h is preferable for power generation than organic removal. Here, the organic removal was referred to as removal per day; however, if the organic removal was calculated in total, then RT 48 h could be better than RT 24. Therefore, the optimum RT of sucrose and acetate could be determined at 24 h and 48 h, respectively. The maximum power generation for sucrose occurred at 24 h, even though a lower COD removal than at 48 h was provided as the accumulation of COD removal increased. Nevertheless, the maximum power generation for acetate occurred at 48 h, which resulted in a higher accumulation of COD removal. To comply with the MFC integrated wastewater treatment for further design, a shorter RT of 24 h is appropriately chosen rather than the longer RT of 48 h. Furthermore, since the adjusted process was mainly in anaerobic conditions, a shorter RT would provide benefits, particularly at the initial/start-up process, capital and operational maintenance (OM) cost, and technical management.

3.4 Summary

The AC-SCMFC batch-mode reactor was used to determine the RT and is worthwhile when designing any MFC reactor type related to integrated MFC compliance for wastewater treatment. Likewise, the diversity of substrate types and concentrations used warrants the determination of recommended substrate type and optimum concentration most suitable for further MFC research in single and multiple MFC chambers. A significant pattern analysis was used to determine which provided a higher pattern for organic removal and power generation factors. In addition, a comparative analysis was performed on eight substantial factors for different substrate types and concentrations to show that they had the most common attention in MFCs studies.

From the results of this study, we recommend acetate substrate for optimum power generation; however, sucrose is preferable for organic removal over a shorter time. Simultaneously, the optimum substrate concentration was a medium level, which showed a significant pattern compared to that of low and high concentrations. In brief, the optimum RT for sucrose was determined to be 24 h and that for acetate was 48 h to fulfill the performance of AC-SCMFC in organic removal and power generation. Considering MFC design and its high overall performance, the optimum RT is 24 h, which applies to both the substrate types and at all levels of organic concentration. We also strongly recommend that the optimum RT obtained from this study could be applied to any type of MFC research, especially in the oxidizable or biodegradable organic range, to ensure high performance. Furthermore, this study was not adjusted for the non-oxidizable or non-biodegradable organic range, which is one of the limitations of MFC studies. Moreover, the different microbial species and diversity applied could affect the performance of MFC. Therefore, we encourage further studies to address the limitations mentioned above to contribute to the results.

Notably, in policy recommendations, we provide the recommended optimum RT with a medium level of substrate concentration and acceptable organic type that can contribute to the design criteria of any MFCs' anodic chamber applied in a large-scale intermittent/continuous mode and ensure that their effluent complies with national and international regulations, standards, and guidelines.

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CHAPTER IV
ENHANCEMENT OF POWER GENERATION AND ORGANIC REMOVAL IN DOUBLE ANODE CHAMBER DESIGNED DUAL-CHAMBER MICROBIAL FUEL CELL (DAC-DCMFC)

4.1 Introduction

Nowadays, energy and water have grown to be the center of the world's attention, particularly for sustainable development. Energy and water support human and living things' activities to produce their demand (Samudro and Mangkoedihardjo, 2020, 2021; Syvitski *et al.*, 2020). Hence, energy and water cannot be separated, due to their synergism creating significant economic and ecological benefits.

A promising approach in facing future challenges is to develop technologies that help human life, stimulate a green environment, and support the world's protocols on sustainable development. Presently, wastewater treatment technologies for organic removal have been reported, from basic to advanced levels, such as adsorption, oxidation, catalysis, photocatalysis, advanced oxidation process, electrochemical oxidation and filtration, membrane bioreactor, anaerobic digestion, etc. (Dominguez *et al.*, 2018; Ahsan, Imam, *et al.*, 2020; Ahsan, Santiago, *et al.*, 2020) However, the cost and technical considerations, especially in operational and maintenance, present a drawback in their application. In addition, more attention has been given to the technologies which provide green by-products, such as methane gas, fertilizer, electricity, water, etc. MFC is a breakthrough technology in electricity generation through a biochemical process that converts organic substrates, which are non-complex, complex, and wastewater, into electricity directly without generator support (Gajda, Greenman and Ieropoulos, 2018; Fadzli, Bhawani and Rania, 2021). This technology could be considered for developing in the future in adopting back-to-nature concepts that use natural organic substrates (Chen *et al.*, 2020; Ahmed *et al.*, 2021), such as wastewater-based biomass, enriching carbons, and nutrients utilized by the electrogenic bacteria (Capodaglio, Bolognesi and Cecconet, 2021; Verma *et al.*, 2021). The research of MFC is still lacking in power generation affected by high internal resistance (Flimban *et al.*, 2019; Paucar and Sato, 2021) and affected by parameters' control (pH, and temperature) (Igboamalu *et al.*, 2019), anolyte (Potrykus *et al.*, 2021), catholyte (Gajda *et al.*, 2020; Lawson *et al.*, 2020; Rossi and Logan, 2020; Waheeb and Al-Alalawy, 2020), inoculum (Nath and Ghangrekar, 2020; Ren *et al.*, 2021), anode (Jingyu *et al.*, 2020; Rajesh, Noori and Ghangrekar, 2020), cathode (Aysla Costa De Oliveira *et al.*, 2020; Chakraborty *et al.*, 2020), electrode spacing (You, Greenman and Ieropoulos, 2018; Singh and Kaushik, 2021), separator (Flimban *et al.*, 2020; Shabani *et al.*, 2020), flowrate (Ieropoulos, Winfield and Greenman, 2010; Krieg *et al.*, 2018), retention time (Santos, de Barros and Linares, 2017), wiring system (Tominaga, Ototani and Darmawan, 2020), design/construction (Miller *et al.*, 2019), and configuration (Aelterman *et al.*, 2006; Clauwaert *et al.*, 2008; Nandy and Kundu, 2018). Design and configuration modifications are studied since the highest internal resistance mostly occurs on the anode chamber.

Most of the design of an MFC reactor is heavily influenced by the retention time, due to the relationship with the loading rate of substrates and reactor volume devoted to fed-batch and continuous processes. Fundamentally, the configurations of MFC consist of a single chamber microbial fuel cell (SCMFC) and double chamber microbial fuel cell (DCMFC) (Nandy and Kundu, 2018). The high performance of DCMFC is shown by the engineered electrode, such as multiple electrodes (Hamed, Majdi and Hasan, 2020; Minutillo *et al.*, 2020), nanomaterial-assisted electrode (Y. Liu *et al.*, 2020; Frattini *et al.*, 2021), low-cost material substituted electrode (Yaqoob *et al.*, 2020; Fatima *et al.*, 2021), separator (Koók *et al.*, 2020; Obileke *et al.*, 2021), and multiple anode chamber called the integrated system with the wastewater treatment plant types (anaerobic digester, constructed wetland, up-flow anaerobic baffled reactor (UASB), etc.) (Kim *et al.*, 2011; Samsudeen *et al.*, 2015; Gajda, Greenman and Ieropoulos, 2018; Abbassi *et al.*, 2020; Capodaglio, Bolognesi and Cecconet, 2021). Multiple anode chamber studies are shown to exhibit greater performance both in power generation and organic removal. The closer type of multiple anode chamber MFC between the previous study with the current study is shown by Kim et al. (2011) (Kim *et al.*, 2011) and Samsudeen et al. (2015) (Samsudeen *et al.*, 2015), in which the results show the power densities of 458 mW/m^2 and 135.4 mW/m^2, respectively. This configuration could be a pioneer of MFC stack or bioelectrochemically-assisted microbial reactor (BEAMR), which produces high power output up to 1.0 V when assisted or integrated with the wastewater treatment plant.

Notably, the multiple anode chamber MFC has not yet been studied extensively on the low-mid concentration of the sucrose-based substrate, graphene nanoplatelets (GNPs) coated anode, and graphene nanoplatelets-water-based polyurethane (GNPs: PU) coated cathode. Hence, the name of the multiple anode chamber in this study is stated as a double anode chamber (DAC) to emphasize its essential functions. The DAC-DCMFC separating the anode chamber into two compartments represents the MFC-assisted wastewater treatment design and configuration to improve its performance and reveals a preferable understanding of the mechanism. Therefore, the objective of this study was to assess the performance of a DAC-DCMFC with varied OLRs, reactor type-based cathodic differences, and two anode chamber compartments, for electrical production and organic removal.

4.2 Materials and Methods

4.2.1 Materials

Mixed bacteria containing electroactive bacteria in sludge form was taken from the Eastern Ube Sewage Treatment Plant (EU-STP), Ube City, Yamaguchi Prefecture, Japan, and placed into the DAC-DCMFC at around 30% of the total reactor volume to stimulate and acclimate to new conditions in a 500 mL anode chamber volume containing the sucrose-based substrate, representing an artificial wastewater solution. Initial sludge treated using a respirometric chamber with 2500 mgCOD/L during 30 days was subsequently analyzed by polymerase chain reaction (PCR) that discovers genetic material as ribonucleic acid (RNA) by amplifying its small amount from EAB specimens into deoxyribonucleic acid (DNA), which is replicated until they are detectable if they exist, denaturing gradient gel electrophoresis (DGGE) that reveals bacteria communities by amplifying 16S ribosomal RNA (rRNA) gene fragments of the PCR product, and sequencing. Universal 16S rRNA gene primer (27 F and 1492 R)–based PCR products from DNA extraction, while universal 16S rRNA gene primers (GC-341 F and 518 R)–based DGGE (Hemalatha, Shanthi Sravan and Venkata Mohan, 2020), were employed to identify the specific species type of mixed bacteria, which have potential as an electrochemically active bacteria (EAB).

Artificial wastewater solution represented by sucrose-based substrates was made of a carbon source and trace elements. Sucrose as a carbon source performing 20,000 mgCOD/l was prepared at 11.86 g per liter added with 8 g per liter of *$NaHCO_3$ 99% p.a.* and *K_2HPO_4 99% p.a.* as the nitrogenphosphate–potassium (NPP) source of trace elements, and other supporting trace elements consisted of 4 milliliters per liter of 2.65 M *$(NH_4)_2HPO_4$ 99% p.a.*, 20 milliliters per liter of 1M *KCl 99.5% p.a.*, 0.4 M *$MgCl_2.6H_2O$ 98% p.a.*, 1.6 M *NH_4Cl 99.5% p.a.*, 0.2 M *$MgSO_4$ 98% p.a.*, 0.16 M *$FeCl_3.6H_2O$ 99% p.a.*, and 0.014 M *$CoCl_2.6H_2O$ 99% p.a.*; and 2 milliliters per liter of 0.68 M *$CaCl_2.2H_2O$ 93% p.a.* (Zhou *et al.*, 2006, 2007). The sucrose-based substrate was varied into three concentrations, which consisted of 400, 1000, and 2500 mgCOD/L diluted from 20,000 mgCOD/L as a stock organic solution, using a dilution equation.

The reactor construction named DAC-DCMFC was built, using acrylic fabrication with the dimension LWH (8.7 × 8.7 × 8.7) cm and thickness 0.3 mm. There were two anode chamber compartments with similar functions. The first and second anode chambers focused on substrate conversion and electron transfer to reveal the new findings regarding the process between those chambers. The proton exchange membrane (PEM) supported by Nafion 117 with the dimension LW (4 × 4) cm was used to separate the second anode chamber and cathode chamber. The anolyte was the sucrose-based substrate with the three concentrations of COD unit, whereas the catholyte was 0.006 M *$Na_2CO_3.1.5H_2O_2$ (CAS# 15630-89-4)* in a 500 mL of cathode chamber volume. Electrodes consisted of *GNPs (CAS# 9002-84-0)*-coated 30 mesh SSM as the anode of both anode chambers, and the big difference was cathode material that consisted of GNPs: PU 1:1 volume solution ratio-coated cathode at the code 1A, and GNPs-PTFE *(CAS# 9002-84-0)* 60% coated cathode at the code 2A. In addition, *synthesized PU* was water-based polyurethane (Ahmad *et al.*, 2020) with a similar function to polytetrafluoroethylene (PTFE) (Ortiz-Martínez *et al.*, 2016; Touach *et al.*, 2016; Fatima *et al.*, 2021) as a waterproofing material and increasing the oxygen reduction reaction (ORR) on the surface of the cathode material. The wiring system, called closed-circuit voltage (CCV) (Marassi *et al.*, 2020; S. H. Liu *et al.*, 2020), connected the anode-equipped 100 Ω of resistor onto the data logger and cathode in a line. In general, the reactor is described in **Figure 4.1**.

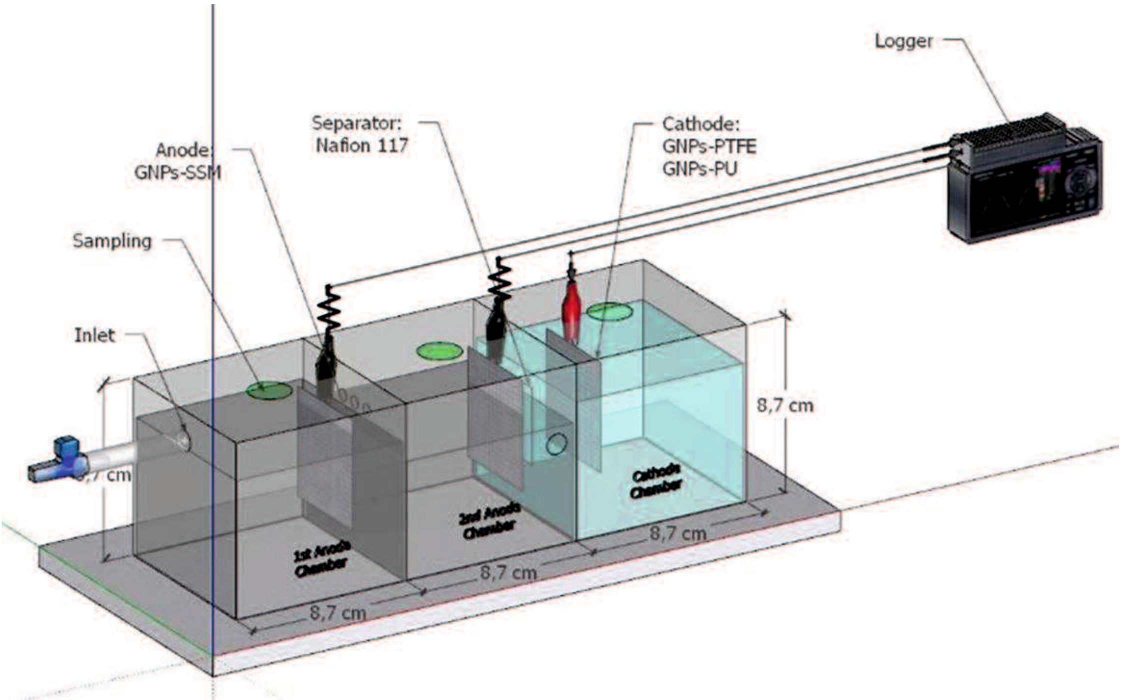

Figure 4.1 Reactor design and configuration.

4.2.2 Methods

4.2.2.1 Practical techniques

The fed-batch mode was run with the support of a peristaltic pump performed by EYELA MP-1000 (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) to manage the stable flow and concentration input of sucrose-based substrate to the reactor and to ensure the anaerobic condition of solution with purging by pure nitrogen gas (N_2) with null oxygen interference.

An amount of sucrose-based substrate prepared in 400, 1000, and 2500 mgCOD/L on 1000 mL of the water tank was injected, using a peristaltic pump to the first anode chamber every 24 h. It can be stated as the organic loading rate (OLR) in 0.4 gCOD/d representing 400 mgCOD/L as low OLR, 1 gCOD/d representing 1000 mgCOD/L as medium OLR, and 2.5 gCOD/d representing 2500 mgCOD/L as high OLR. These level-strength wastewaters can be found at Stoll et al. (2018) (Stoll, Dolfing and Xu, 2018). The solution of the first anode chamber passed to the second anode chamber after injecting it to the first anode chamber was done within 20 min. After leaving it for 24 h; then, the sample from the first and second anode chamber was taken around 10 mL for analyzing OD_{600}, COD, and TOC, and prepared for preservation using the refrigerator at 4 °C for less than 24 h. The physical parameters, such as pH, temperature, and ORP, were analyzed immediately after the 24 h running process, whereas the power in the voltage unit for every reactor was recorded every 30 min. The sampling time was determined at steady-state every 24 h over 192 h, which was previously acclimatized within two months for every reactor type, anode compartments, and OLRs to investigate their effect. Likewise, a brief explanation of the DAC-DCMFC setup is shown in **Table 4.1**.

Table 4.1 Set up of the DAC-DCMFC component based on the variables used.

Reactor Type	Anolyte	Anode		Separator	Catholyte	Cathode
		A1	A2			
1	Sucrose-based substrate	GNPs-SSM 30 mesh	GNPs-SSM 30 mesh	CEM (Nafion 117)	2 g/L $Na_2CO_3.1.5H_2O_2$	GNPs:PU 1:1(*v*/*v*)-SSM 30 mesh
2	Sucrose-based substrate	GNPs-SSM 30 mesh	GNPs-SSM 30 mesh	CEM (Nafion 117)	2 g/L $Na_2CO_3.1.5H_2O_2$	GNPs:PTFE 60%-SSM 30 mesh

Here, based on **Table 4.1** above, all the captions in the figures afterward are defined as follow: 1A1 and 1A2: 1st anode-GNPs-SSM, 2nd anode-GNPs-SSM, and cathode-GNPs: PU-SSM; 2A1 and 2A2: 1st anode-GNPs-SSM, 2nd anode-GNPs-SSM, and cathode-GNPs: PTFE-SSM; X: low OLR; Y: medium OLR; Z: high OLR.

4.2.2.2 Analytical techniques

The physical parameters consisted of pH, temperature, ORP, and OD_{600}. The pH and temperature in degree Celsius (°C) were performed by pH meter D-72 HORIBA Advanced Techno Co., Ltd., Kyoto, Japan). ORP in millivolt (mV) was carried out by pH/ORP meter D-72 (LAQUA act, HORIBA, Japan). OD600 in absorbance (Abs) was operated at wavelength 600 nm by spectrophotometer U-2900 (Hitachi High-Tech Science Corporation, Tokyo, Japan). The chemical parameters consisted of COD and TOC, both in milligram per liter (mg/L). COD and TOC were executed through 5220 C, closed reflux, titrimetric method, and 5310 B, high-temperature combustion method, respectively, by Standard Methods For The Examination Of Water And Wastewater 23rd edition (APHA, AWWA and WEF, 2017). Specifically, the COD instrument analysis was supported by block heater MG-2200 (Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and the TOC analysis was operated by TOC-V Shimadzu (Shimadzu Corporation, Kyoto, Japan).

The power parameter in the voltage unit was performed by the data logger, midiLOGGER GL-240 (Graphtec Corporation, Yokohama, Japan), with a sampling time every 30 min.

4.2.2.3 Data calculation

The percentage of COD removal was counted with the following formula: %COD removal = $\frac{COD_{in}-COD_{out}}{COD_{in}} \times 100\%$, where COD in (mg/L): the COD of the influent; COD out (mg/L): the COD of the effluent (Kloch and Toczylowska-Maminska, 2020). The TOC removal was calculated by using the analogical COD removal formula as follows: TOC removal = $\frac{TOC_{in}-TOC_{out}}{TOC_{in}} \times 100\%$, where TOC_{in} (mg/L): the TOC of the influent; TOC_{out} (mg/L): the TOC of the effluent.

Power and current were enumerated by following Ohm's law: V = I.R; P = V.I; where V (volt) is power; P (watt) is power; I (ampere) is current; and R (ohm) is resistance. Power density (PD), volumetric PD and coulombic efficiency (CE) were computed by the following formula: PD = (V.I)/A and PD = (V.I)/WV, where V (volt): power; I (ampere): current; A (m^2): anode surface area; WV (m^3): working volume (Logan, 2008). CE = $\frac{M.\int_0^t I.dt}{n.F.V_{anodic}.r_{COD}}$, where M: the oxygen molar mass (32); I: the electrical current intensity utilized by the MFC (Ampere.second); n: the actual electrons transferred (4 mmol of electron per mmol of COD); F: the Faraday constant (96485 C/mol electrons); Vanodic: the volume of anodic (L), rCOD: the removal rate of COD (g/L) (Logan, 2008).

4.3 Results

4.3.1 Seeding and Feeding Characteristics

Seeding, which means an EAB source from the sludge of EU-STP, was initially characterized to acquire its condition and to ensure that no other nuisances were affected by the other possible parameters. The results were as follows: pH 7.1 ± 0.04, temperature 20 ± 5.0 °C, biochemical oxygen demand (BOD) 198 ± 2 mg/L, COD 396 ± 2 mg/L, TOC 57 ± 1 mg/L, total phosphorous in phosphate (P-PO_4) 266 ± 5 mg/L, total nitrogen in nitrate (N-NO_3) 26 ± 5 mg/L, total solids (TS) 12740 ± 140 mg/L, total suspended solids (TSS) 11973 ± 690 mg/L, volatile suspended solids (VSS) 8415 ± 420 mg/L, and fixed suspended solids (FSS) 3320 ± 175 mg/L. The sequence analysis results using the Basic Local Alignment Search Tool Nucleotide (BLASTN) National Center for Biotechnology Information (NCBI) with the similarity results up to 96% consisted of *Lactococcus laudensis, Schwartzia succinivorans, Selenomonas lacticifex, Arcobacter lekhitochrous, Arcobacter butzleri, Clostridium polynesiense,* and *Clostridium aurantibutyricum*.

Feeding, which means a sucrose-based substrate, was characterized in pH, temperature, ORP, OD600, COD, and TOC as the primary tested parameters during the operational period, based on the different OLRs. The results are listed in **Table 4.2**.

Table 4.2 Feeding characterization in average

Parameters	Low OLR	Medium OLR	High OLR
pH	8.8 ± 0.09	8.3 ± 0.1	7.6 ± 0.01
Temperature (°C)	24.7 ± 1.09	24.2 ± 0.34	24.2 ± 0.12
ORP (mV)	−226 ± 3.98	−306 ± 3.98	−255 ± 7.74
OD_{600} (Abs)	0.025 ± 0.007	0.04 ± 0.011	0.5 ± 0.29
COD (mg/L)	407 ± 68.89	1040 ± 160	2387 ± 205
TOC (mg/L)	87 ± 2.08	230 ± 4.65	724 ± 40

4.3.2 Parameters Changes

This observation during the time-frame limitation can be correlated to find the differences among them, particularly the different effects among reactor type and two anode chamber compartments with the different OLRs.

Figure 4.2 shows that those pHs of the low, medium, and high OLRs at the first and second anode chambers in type 1 and type 2 reactors were in the range of 6 to 8 during an operational period of 192 h. The initial pHs of each input of OLRs denoted differently in which from low OLR to high OLR presented 8.8, 8.3, and 7.6, respectively. The inside pH in both reactors pointed, on average, to 6.6 ± 0.07 of low and medium OLRs, and to 6.1 ± 0.01 of high OLR. Meanwhile, temperatures of the low, medium, and high OLRs at the first and second anode chambers in type 1 and type 2 reactors had no substantial difference during a running period of 192 h. The initial temperatures of each input of OLRs showed approximately 24.4 ± 0.3 °C. The inside temperatures in both reactors denoted, on average, 26.7 ± 0.2 °C of low and high OLRs, and 24 ± 0.1 °C of medium OLR. Whereas ORPs of the low, medium, and high OLRs at the first and second anode chambers in type 1 and type 2 reactors were in the range of −299 to −356 mV during an operational period of 192 h. The initial ORPs of each input of OLRs denoted differently in which from low OLR to high OLR presented −226 mV; −306 mV; −255 mV, respectively. The inside ORP in both reactors pointed on average −320 ± 11 mV, −351 ± 6 mV, and −319 ± 14 mV of the low, medium, and high OLRs, successively. Further, OD_{600}s, CODs, and TOCs of the low, medium, and high OLRs at the first and second anode chambers in type 1 and type 2 reactors had significant differences over an operational period of 192 h. The initial OD600s of each input of OLRs were denoted differently for which low OLR to high OLR presented 0.025 Abs, 0.04 Abs, and 0.52 Abs, serially. In succession, the inside OD_{600} in both reactors pointed on average to 0.226 ± 0.06 Abs, 0.296 ± 0.03 Abs, and 0.544 ± 0.02 Abs of low, medium, and high OLRs, whereas the initial CODs of each input of OLRs were denoted differently for which low OLR to high OLR presented 407 mg/L, 1080 mg/L, and 1118 mg/L, respectively. The inside COD in both reactors pointed, on average, to 242 ± 24 mg/L, 360 ± 60 mg/L, and 1118 ± 241 mg/L of the low, medium, and high OLR, serially. Meanwhile, the initial TOCs of each input of OLRs were denoted differently for which low OLR to high OLR presented as 87 mg/L, 230 mg/L, and 334 mg/L, successively. The inside COD in both reactors pointed, on average, to 25 ± 5 mg/L, 58 ± 21 mg/L, and 184 ± 65 mg/L of the low, medium, and high OLR, respectively.

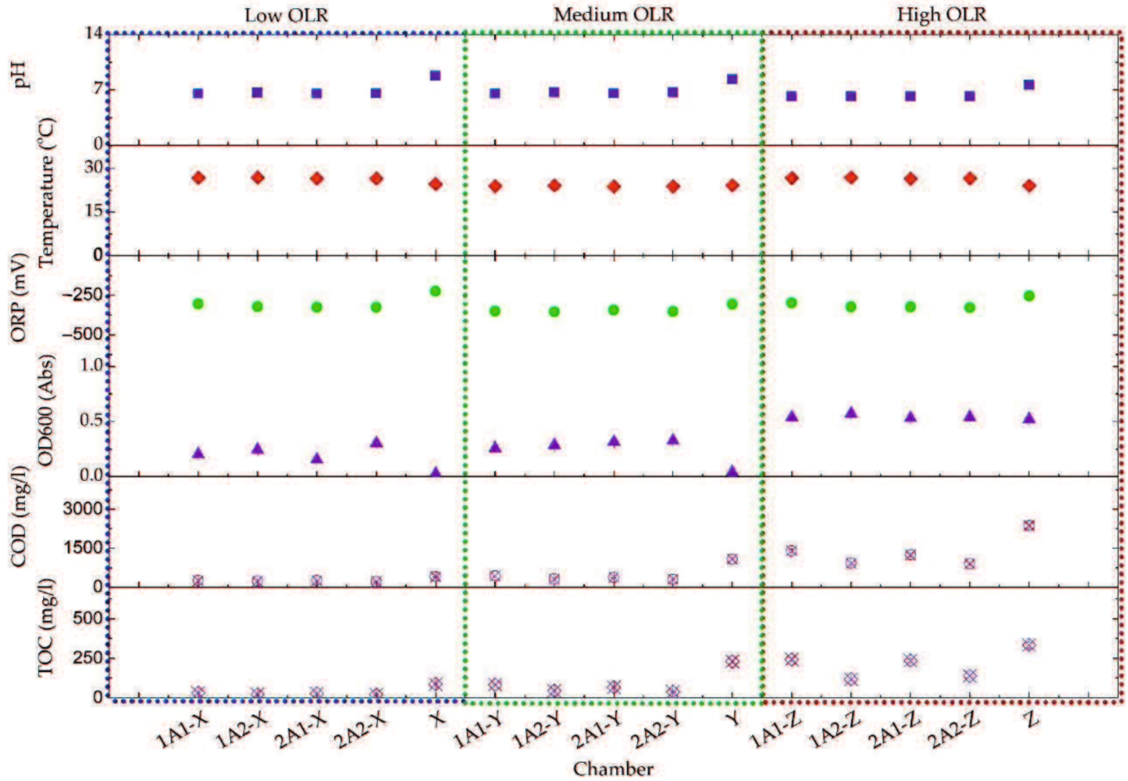

Figure 4.2 Parameters level changes comparison among OLRs; X: Low OLR; Y: Medium OLR; Z: High OLR; 1A1: type 1 reactor and first anode chamber; 1A2: type 1 reactor and second anode chamber; 2A1: type 2 reactor and first anode chamber; 2A2: type 2 reactor and second anode chamber.

4.3.3 COD and TOC Removal

Generally, **Figure 4.3** shows that the COD and TOC removal maximum of medium OLR at the first and second anode chamber in type 1 and type 2 reactor were more stable at around 60–80% than COD and TOC removal maximum of low and high OLRs. Low OLR presented the COD and TOC removal maximum at the first and second anode chamber in the type 1 and type 2 reactor at around 10–80%, whereas high OLR performed COD and TOC removal maximum at the first and second anode chamber in type 1 and type 2 reactor at around 40–80%.

The COD concentration of low and high OLR at the first and second anode chamber was removed at the same level in which the only difference was the TOC concentration utilization. The TOC concentration of low OLR noticed a decrease at the second anode chamber but higher usage at the first anode chamber. At the same time, the TOC concentration of high OLR appeared to increase at the type 1 and 2 reactors.

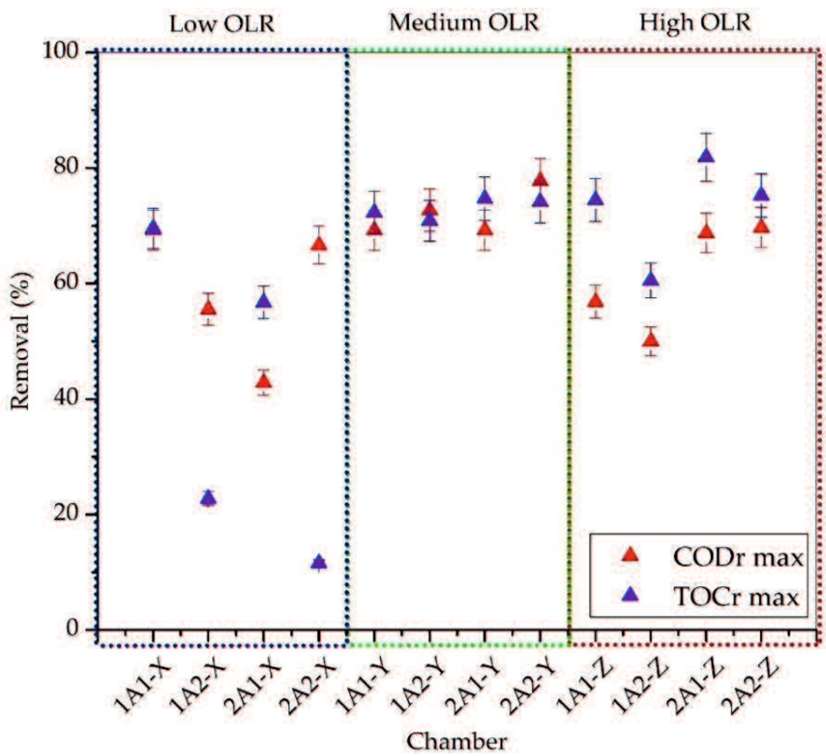

Figure 4.3 COD and TOC removal among OLRs; X: low OLR; Y: medium OLR; Z: high OLR; 1A1: type 1 reactor and first anode chamber; 1A2: type 1 reactor and second anode chamber; 2A1: type 2 reactor and first anode chamber; 2A2: type 2 reactor and second anode chamber.

4.3.4 Power Production

The acclimation was operated within 1483 h or 62 days to provide stable power output during the injection afterward. The acclimation process showed that both reactors at the first and second anode chambers had different patterns.

Figure 4.4 shows that the first anode chamber of both reactors started increasing from minus power due to adaptation with new concentrations input at time zero to around +114 mV of the type 1 reactor and at 122.5 h to about +264 mV of the type 2 reactor. Furthermore, the powers significantly increased to around +400–500 mV at 797 h for the type 1 reactor and 576.5 h for the type 2 reactor. It can be stated that the type 2 reactor provided a faster adaptation to the new concentration input than the type 1 reactor, where both were in the first anode chamber. The power of the reactor type 1 and 2 became well built around +400–500 mV until 1446.5 h right before injecting with the different OLRs variable.

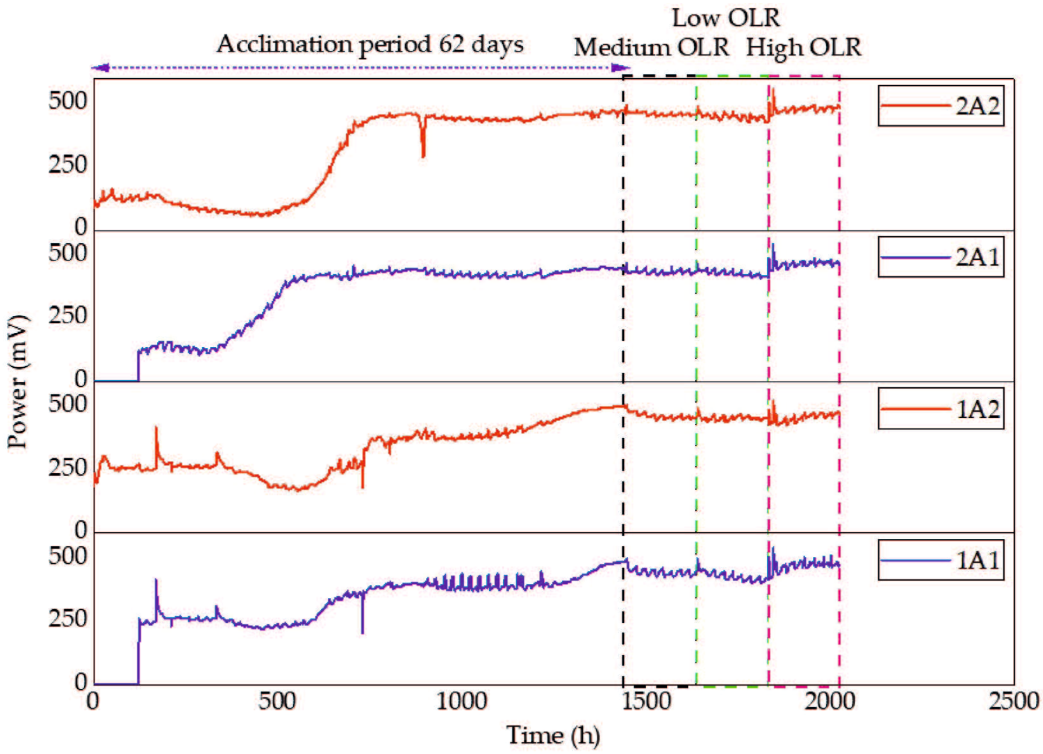

Figure 4.4 The overall power production; 1A1: type 1 reactor and first anode chamber; 1A2: type 1 reactor and second an-ode chamber; 2A1: type 2 reactor and first anode chamber; 2A2: type 2 reactor and second anode chamber.

Whereas the second anode chamber of both reactors started at around +200 mV and +100 mV at the beginning of acclimation, the power reached +400 mV for the type 1 reactor at +874 mV and the type 2 reactor at +705 h. It was similar to the first anode chamber aforementioned that the type 2 reactor provides a faster power increase than the type 1 reactor. After the acclimation process, the power was stable at around +400–500 mV.

At a glance, the power outputs were much the same level, but the difference between them could be identified clearly. At low and medium OLRs, the powers were around +400–500 mV; meanwhile, at high OLR, the power was around +400–550 mV. There was a difference of 50 mV higher at high OLR compared to low and medium OLRs.

Figure 4.5 shows that power production performed very stably at a maximum of 483.3 ± 15 mV among OLRs, reactor types, and anode chamber compartments. The power density at the second anode chamber showed to be relatively less high than at the first anode chamber among OLRs; however, the level presented was similar at a maximum of 866 ± 44 mW/m^2. Meanwhile, the volumetric power density reached a maximum of 5.15 ± 0.26 W/m^3. Interestingly, CE achieved a maximum of 83% of low OLR, 84% of medium OLR, and 53% of high OLR. The level of CE showed more than

100%, which could occur when the system was started at the beginning of the process, starting from 0 h to less than 120 h of observation.

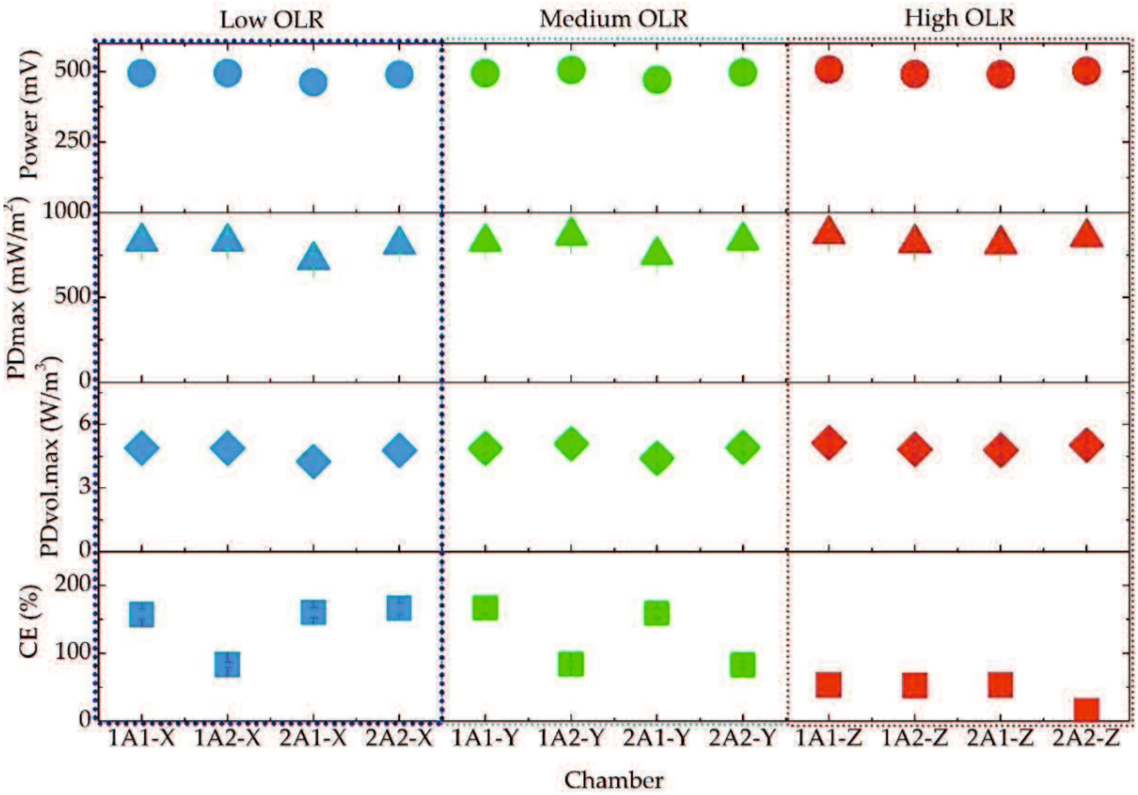

Figure 4.5 Power density (PD) and coulombic efficiency (CE); X: low OLR; Y: medium OLR; Z: high OLR; 1A1: type 1 reactor and first anode chamber; 1A2: type 1 reactor and second anode chamber; 2A1: type 2 reactor and first anode chamber; 2A2: type 2 reactor and second anode chamber.

4.4 Discussion

4.4.1 Conceptualization

One of the exciting schemes is treating the wastewater containing a high organic loading in an anaerobic digester (AD). Subsequently, a low organic loading output of AD is treated by stack MFC system or BEAMR to produce a very low organic loading to meet the water effluent standard and high-power production at the end of treatment (Aelterman *et al.*, 2006; Clauwaert *et al.*, 2008). Hence, this study is structured more closely with the integrated microbial fuel cell-wastewater treatment (MFC-WWT) in which the first anode chamber has the same function as wastewater treatment and the second anode chamber has the function of transferring abundantly generated electrons from the first anode chamber; even the process is similar to the first anode chamber.

In addition, since the first anode chamber has a similar function with anaerobic treatment, therefore, the additional information related to the anaerobic treatment could be represented by the F/M ratio to ensure that the process follows the standard of the anaerobic process. The F/M ratio means food-to-microorganism organic loading per time divided by the number of microorganisms represented by volatile suspended solids (VSS). Since this study used three different OLRs, the F/M ratio can subsequently be obtained by dividing OLR toward the existing VSS. The F/M ratio from low OLR to high OLR is 0.1, 0.24, and 0.59 gCOD/gVSS.d, respectively. The F/M ratio should be in the range of 0.45–0.50 to efficiently solubilize cells (Tanaka *et al.*, 1997). The best value of F/M ratio is between 0.57and 0.68 for anaerobic digestion (Prashanth, Kumar and Mehrotra, 2006). The F/M ratio was found to be optimal at 0.5 for biogas production in an anaerobic digester (Gözde Tuğba Köksoy, 2009). Since the MFC has a function to generate electricity, which can be inhibited by high gas production from methanogenic bacteria, then the optimal F/M ratio should be less than 0.5 gCOD/gVSS.d. In correlation with the recommended optimal OLRs in this study, the optimal F/M ratio can be determined at 0.24 gCOD/gVSS.d to generate more electricity with less gas production.

4.4.2 Seeding and Feeding

The seeding characteristics showed a good strength concentration level of several identified parameters that some EABs could grow and adapt very well. According to the mentioned sequence results, the potential EABs were determined to ensure and support the performance of DAC-DCMFC. *Arcobacter lekhitochrous* and *Arcobacter butzleri,* a part of the Arcobacter group, are defined explicitly as epsilon proteobacteria (ε-proteobacteria), which is classified as one of the proteobacteria genera, having a function of electron producing and transferring active bacteria (Fedorovich *et al.*, 2009; Toh *et al.*, 2011; Pereira-Medrano *et al.*, 2013; Szydlowski *et al.*, 2020). Likewise, *Clostridium polynesiense* and *Clostridium aurantibutyricum,* a part of the *Clostridium* group, are particularly defined as firmicutes that are stated to be one of EABs, having a similar function with ε-proteobacteria. At the same time, *Lactococcus laudensis*, *Schwartzia succinivorans*, and *Selenomonas lacticifex* are synergistic as a catalyst to convert organic substances to the electron. *Schwartzia sp.* and *Selemonas sp.,* specified as anaerobic, Gram-negative, and non-spore-forming bacteria, are the most closely related genera (Van Gylswyk, Hippe and Rainey, 1997). *Lactococcus sp.* is a Gram-positive bacteria that utilize the soluble redox mediators taken by Gram-negative bacteria (Rabaey and Verstraete, 2005; Pham *et al.*, 2008).

Regarding the feeding characteristics in **Table 4.2**, the results indicate that the pH showed to be lower when OLR became higher, but the pH of feeding was still neutral, which was useful for the adaptation of microorganisms to the new environment which has a pH range of 5–8.5 (Hilton *et al.*, 2001). Only at the low OLR would the optimal living of microorganisms be inhibited. The temperature also showed a good environmental factor that is adaptable for microorganism living, especially for the seeding characteristics, which can live optimally around 15–37 °C (Hilton *et al.*, 2001). The overall ORP value was appropriately performed due to the pre-treatment using nitrogen gas injection into the solution. The ORP was reported in a range of −200 mV to −100 mV in performing the integrated MFC-CW(Yan *et al.*, 2018), the bio-electrochemical oxidation of sulfide utilized by sulfate-reducing bacteria (SRB) has an ORP range of −150 mV to −100 Mv (Ryckelynck, Stecher III and Reimers, 2005), and the phosphorus (P) utilization and energy production in the dual chamber-microbial fuel cells (DCMFCs) presented an ORP range of −600 mV to −300 mV (Almatouq and Babatunde, 2016). Therefore, ORP could be an option for process monitoring in MFC related to environmental factors. Subsequently, OD_{600} shows that it could be higher at high OLR. It could be depicted here that high OLR in the COD unit affected the OD_{600} value and would influence the end value of OD_{600}.

In addition to these feeding characteristics, the effect of the presence of organic pollutants and inorganic pollutants known as complex substrates on the performance of MFCs has been reported in many MFC studies. Specific components derived from salt (chloride) (Miyahara, Kouzuma and Watanabe, 2015; Grattieri and Minteer, 2018), and heavy metals (chromium, cadmium, mercury, lead, etc.) (Adekunle, Raghavan and Tartakovsky, 2019; Gustave *et al.*, 2021; Sharma *et al.*, 2021) are the parameters that most inhibit MFC performance. Since this study used a pure substrate containing components and concentrations suitable for EAB, this substrate did not affect its performance.

4.4.3 Factors Affecting the Performance

The pH, temperature, and ORP used in this research were defined as the control parameters to manage the applied variables. pH is an imperative parameter that affects the performance of DAC-DCMFC since the EAB typically comprise adaptive microorganisms at pH 6–8 (Puig *et al.*, 2010). The results presented that the input pH of different OLRs and observed pHs during DAC-DCMFC operation could successfully stimulate the EABs to produce and transfer electricity. The pH input of the different OLRs between 7 and 9 could help the system suit the environmental condition and prevent the shock loading in which pH 8–10 was reported to improve the electrical generation in MFC (Liu *et al.*, 2010). Various references reported that the observed pH between 6.8 and 7.5 is the optimal range for methanogenic activities, meaning operation in an anaerobic condition (Li, Chen and Wu, 2019). The pH would affect CE (Kaur *et al.*, 2014; Rajesh, Jadhav and Ghangrekar, 2015), CH_4 production (Li, Chen and Wu, 2019), the release of CO_2 (Geng *et al.*, 2020), the anode reaction kinetics (Yuan *et al.*, 2011; Liao *et al.*, 2014), the electrochemical relationship between anode and biofilm (Liao *et al.*, 2014), the population density of EAB (Igboamalu *et al.*, 2019), suppress or inactive the methanogenic bacteria, which is currently known to be contributing to one of the EAB (Igboamalu *et al.*, 2019), the electroactive moiety density and the main effect is reducing the MFC internal resistance (Yuan *et al.*, 2011).

At this point, the significant effect of pH on the other observed variables was OD_{600}, COD, TOC, and power production. OD_{600} presenting the density of bacteria population would increase significantly in which the pHs were in the optimal range value, whereas COD and TOC representing the organic substances containing carbon and nutrients, would be removed substantially as well (Gonzalez del Campo *et al.*, 2013; Mateo *et al.*, 2016). The power production could be stable during the process and directly improve the entire MFC performance (Mahmood *et al.*, 2017). Owing to the fact that pH, temperature, and ORP were not the independent variables, subsequently, the effect was not too considerable. Those variables were only observed during the process to ensure that the condition was in the appropriate range.

Based on the observation of both the type 1 and 2 reactors, as seen in **Figure 4.2**, pHs at the first anode chamber were no significant different than at the second anode chamber since the first anode chamber contained a high concentration of COD and TOC that affected the decreasing of pH, due to the high hydrogen ion generation (López Velarde Santos *et al.*, 2017; Algar *et al.*, 2020). There were no such different values between the type 1 and 2 reactors of each OLR.

Linares et al. (2019) (Linares *et al.*, 2019) reported that COD removal in several studies achieved ranges from 36% to 84%. It could be said that the results of this study are still on the normal performance of MFC research, even though the maximum COD removal could achieve up to 78%. In comparison with the similar condition of this study, COD removal is around 58% during 48 h with a power maximum at 498 mV and current maximum at 7.9 mA in CCV mode (Hemalatha, Shanthi Sravan and Venkata Mohan, 2020).

Particularly, it could be stated for a type 1 reactor that higher OLR does not mean higher COD removal. However, it could be appointed on the type 2 reactor that the higher the OLR, the higher COD removal is linearly achieved. Santos et al. (2017) (López Velarde Santos *et al.*, 2017) reported that higher-applied COD concentration influencing the OLR could decrease the COD removal. The other study reported similar results in which treating swine wastewater at a high concentration of COD decreased the COD removal (Goto and Yoshida, 2019). When undiluted wood industry wastewater applied on SMFC, the COD removal only achieved a maximum of 40% (Kloch and Toczylowska-Maminska, 2020). This result is appropriate with the applied OLR recommendation between 0.05 $kg.m^{-3}.d^{-1}$ and 2 $kg.m^{-3}.d^{-1}$ for optimizing any kind of MFC system by many types of research (Scott *et al.*, 2012).

Likewise, it could be stated that the COD removal at the first and second anode chamber on both reactors is relatively similar, as seen in **Figure 4.3**. In comparison, medium and high OLR appeared to be the same or slightly different on COD removal, even though high OLR still provided high COD removal. It could be concluded that the microbial activities are adaptable in high COD concentrations.

Meanwhile, the TOC removal maximum contributes similarly to the COD removal maximum, as seen in **Figure 4.3**. Medium and high OLR provides up to 100% TOC removal meaning that the carbon source as the organic carbon is properly utilized by the EAB. It could be concluded to be same as the COD removal. This study of organic carbon utilization in MFCs has not been yet comprehensively studied to investigate the correlation between COD and TOC in which TOC is a component part of COD used by EAB as their primary carbon source.

Based on the calculation of the final COD concentration in daily averages of the three OLRs levels, the treated effluent from the low and medium OLRs of the DAC-DCMFC meets the 2015 Japanese National Effluent Standard (J-NES), where the COD level is set to a maximum of 120 mg/L for a daily average, whereas the high OLR does not meet J-NES 2015. This study opens up further opportunities and challenges, especially for dealing with high OLR, that a multi-anode chamber known as anode baffle chamber for multi-stage treatment can be developed to gradually enhance the organic removal and ensure an increase in power generation.

In addition, COD and TOC removal were revealed at different OLRs and each anode chamber compartment. From this information, we can calculate the further design and construction of the DCMFC. Because the design criteria for the multi-anode compartment are not concise, this study contributes to its use as a benchmarking design.

4.4.4 Power Production Performance

Figure 4.5 shows that low OLR performed lower than medium and high OLRs in power production. Likewise, the type 1 and 2 reactors demonstrated similarly in power production at the overall OLRs level. Furthermore, the second anode chamber presented higher power production at overall OLRs than the first anode chamber. In fact, the second anode chamber enhanced the power production to be 3–6% higher than the first anode chamber both in type 1 and 2 reactors. Moreover, the second anode chamber was two-fold in HRT or 24 h longer than the first anode chamber; hence, an abundant electron that passed from the first anode chamber could be transferred to the second anode chamber. Another reason is the cathode material used that contributes to increasing the ORR rate on the surface of the cathode (Cheng, Liu and Logan, 2006; Zhang *et al.*, 2016; Midyurova and Nenov, 2017; Walter, Greenman and Ieropoulos, 2018).

As seen in **Figure 4.4** and **Figure 4.5**, the power production showed approximately on average at 460 mV, when the highest was achieved at 483 mV. The difference in power was only less than ±5%. Consequently, the power production among OLRs level could be defined similarly. It is comprehensible in which the measurement was conducted in a stable position after the acclimation period.

Additionally, a comparison study was conducted to support the explained information above regarding the associated references in power production. This study used the components mentioned in **Table 4.1**, and the results provided power at a maximum of 483.3 ± 15 mV with power density at a maximum of 866 ± 44 mW/m^2 (5.15 ± 0.26 W/m^3), and CE reached a maximum of 84%. Meanwhile, other studies that used lactate as the anolyte, air as the catholyte, solid graphite rods as the anode and cathode, and PEM Nafion Alfa Aesar presented a power density at a maximum of 458 mW/m^2 (23.6 W/m^3) (Kim *et al.*, 2011). The other studies using a different anolyte from real distillery wastewater, a catholyte from potassium ferricyanide, and an electrode from graphite provided a power density at a maximum of 135.4 mW/m^2 (368 mA/m^2) (Samsudeen *et al.*, 2015). Coupled with related references performed by DA-MFC, which used real brewery wastewater as an anolyte, air as a catholyte, graphite felt as an anode, graphite felt loaded Pt/C as a cathode, and was membraneless presented, at a maximum, a power of 0.46 mW (Kim *et al.*, 2016). All related studies showed high performance on a couple of sides, such as high volumetric power density, which means a high predictor of in-field application. This study is located on the high level of power density, indicating that this configuration has increased the power density at a maximum of 1.9-fold rather than the previous studies.

Meanwhile, CE achieved a maximum of 84% measured after 144 h since the previous CE showed up to 100%. These CE ranges are acceptable as the minimum requirement of at least 80% to optimize the MFC system (Stoll, Dolfing and Xu, 2018). In comparison with the other studies using acetate as their substrate, CE achievement was up to 70% (Rozendal *et al.*, 2007; Sleutels *et al.*, 2011).

However, the volumetric power density was still lower than the other studies associated with general DCMFCs. It could happen since the design volume of the anode chamber did not adopt the stack MFC. The other studies performed analytical MFC design using disposable polypropylene as their findings to adjust anode distance to the membrane or anodic volume without redesigning the MFC construction many times (You, Greenman and Ieropoulos, 2018). Nevertheless, we could understand and reveal that volumetric power density can be enhanced by modifying the anode chamber. Here, we can contribute to the knowledge regarding the optimum design of future multiple anode chamber MFC and strengthen the cognition in developing multiple anode chambers into stack MFC integrated with WWTPs.

4.4.5 Cost Consideration

This study used two types of reactors with different cathodic materials. In order to face further practical use, a cost analysis was calculated to compare the appropriate design and configuration applied in the field. The specific cost analysis is the cost-benefit ratio (C/B). The cost component consists of capital and O&M costs, while the benefit component consists of returning or recovering the use of the reactor. Disbenefit is the effect of using the reactor. For the purpose of calculating the C/B ratio, the inflation assumption is set at 6% per year with a viable economic period of 10 years.

Based on the analysis, the C/B ratio of the type 1 reactor is 0.7 less than the recommended one of 1 so that it provides advantages in its application. Compared to the C/B ratio of the type 2 reactor, which is 1.3 over 1, it presents a small advantage. It is the case where the type 2 reactor used an expensive catalyst to obtain the ORR of the cathodic material. While the type 1 reactor used an alternative catalyst that was used as waterproofing as well, the costs incurred are only 2% of the catalyst used in the type 2 reactor, providing stable and the same organic removal as the type 2 reactor.

4.5 Summary

DAC-DCMFC is a promising design and configuration of MFC technology in power production and organic pollutant removal. It contributes to increasing power density 1.9 times, compared to the closer design and configuration of other multiple anode chambers MFC reported in the literature. Power production was achieved at a maximum of 866 ± 44 mW/m^2 (5.15 ± 0.26 W/m^3), and coulombic efficiency (CE) reached a maximum of 84%. COD and TOC removals represented by medium OLR achieved the maximum organic removal ranging from 60% to 80%.

There were no significant differences among low, medium, and high OLRs in power production; however, the different levels of OLRs highly influenced the COD and TOC removal in which low OLR presented as being lower than medium and high OLRs. Meanwhile, the second anode chamber achieved higher performance in power production at overall OLRs than the first anode chamber. In addition, there were no substantial differences in COD and TOC removal between the two compartments.

Overall tested OLRs could be used for further study in the development of DAC-DCMFC. For recommendation, medium OLR is more preferred for the start-up process, due to fast EAB adaptation and stable power output in long-term performance. The modified anode chamber by separating the anode space into two parts in a dual anode chamber microbial fuel cell applied by different organic loadings reveals a better understanding of the actual application of a wastewater treatment integrated microbial fuel cell.

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CHAPTER V
CONCLUSIONS AND FUTURE WORKS

5.1 Conclusions

Below are the conclusions obtained from this study:

- The optimum RT, performed by the AC-SCMFC batch-mode reactor, is determined at 24 h, which applies to both the substrate types and at all levels of organic concentration by considering MFC design and its high overall performance. In addition, the optimum RT obtained from this study could be applied to any type of MFC research, especially in the oxidizable or biodegradable organic range, to ensure high performance.

- Power production, indicated contributing to increasing power density 1.9 times, compared to the closer design and configuration of other multiple anode chambers MFC reported in the literature, was achieved at a maximum of 866 ± 44 mW/m^2 (5.15 ± 0.26 W/m^3), and coulombic efficiency (CE) reached a maximum of 84%. Meanwhile, COD and TOC removals represented by medium OLR achieved the maximum organic removal ranging from 60% to 80%. Furthermore, the second anode chamber achieved higher performance in power production at overall OLRs than the first anode chamber. Moreover, there were no substantial differences in COD and TOC removal between the two compartments.

- This study recommends acetate substrate for optimum power generation; however, sucrose is preferable for organic removal over a shorter time. Simultaneously, the optimum substrate concentration was a medium level, which showed a significant pattern compared to that of low and high concentrations.

- This study suggests that medium OLR is more preferred for the start-up process, due to fast EAB adaptation and stable power output in long-term performance. Furthermore, based on the cost consideration, the C/B ratio of the type 1 reactor is 0.7 less than 1, providing advantages in its application.

- Remarkably, the recommended optimum RT, resulted from AC-SCMFC, with a medium level of substrate concentration and acceptable organic type that can contribute to the design criteria of any MFCs' anodic chamber applied in a large-scale intermittent/continuous mode and ensure that their effluent complies with national and international regulations, standards, and guidelines. Likewise, based on the performance of DAC-DCMFC, the modified anode chamber by separating the anode space into two parts in a dual anode chamber microbial fuel cell applied by different organic loadings reveals a better understanding of the actual application of a wastewater treatment integrated microbial fuel cell.

5.2 Future Works

Suggestions for future research topics concerning the simultaneously enhanced organic removal and power generation in the next MFC studies based on multi-chamber MFCs are listed below:

- The condition of the reactor indicated anoxic-like anaerobic; this study suggests strictly setting the reactor at obligate anaerobic to identify the different performances under the same conditions and contribute to comparing their results afterwards.
- Based on the finding of optimum RT, further innovations can be obtained by designing multi-compartment-based anodic chambers assisted with single or multi-compartment-based cathodic chambers to improve the performance of multi-chamber-based MFCs.
- The concentration and OLR of medium strength levels could benchmark all MFC studies to quickly start up the reactor's operation and ensure high performance both in organic removal and power generation that complies with the national and international standards of discharge and MFC guidelines.
- This study encourages further research to address the limitations of the range of non-oxidizable or non-biodegradable organics and the different microbial species and diversity that may contribute to the results.

APPENDIX

List Publications

1. Enhancement of Power Generation and Organic Removal in Double Anode Chamber Designed Dual-Chamber Microbial Fuel Cell (DAC-DCMFC) – Journal: Water (Switzerland) year 2021, Authors: **Ganjar Samudro**, Tsuyoshi Imai, and Yung-Tse Hung; https://doi.org/10.3390/w13212941 (research study in Yamaguchi University period 2019 - 2022)
2. Determination of optimum retention time in an air–cathode single-chamber microbial fuel cell batch-mode reactor by comparing different substrate types and concentrations - Journal: Process Safety and Environmental Protection year 2022, Authors: **Ganjar Samudro**, Tsuyoshi Imai, and Alissara Reungsang; https://doi.org/10.1016/j.psep.2022.04.023 (research study in Yamaguchi University period 2019 - 2022)
3. Comparison of leachate and mixed waste generated electricity in Compost Solid Phase Microbial Fuel Cells (CSMFCs) - IOP Conference Series: Earth and Environmental Science year 2021, Authors: **Ganjar Samudro**, Syafrudin, Irawan Wisnu Wardhana, and Tsuyoshi Imai; https://doi.org/10.1088/1755-1315/623/1/012098 (research study in Indonesia)
4. Comparison of Waste Volumes on Power Generation and COD Removal in Solid Phase Microbial Fuel Cell (SMFC) - Journal: IOP Conference Series: Earth and Environmental Science year 2019, Authors: **Ganjar Samudro**, Pertiwi Andarani, Winardi Dwi Nugraha, Irawan Wisnu Wardhana, Sarwoko Mangkoedihardjo, Hilma Muthi'ah, Glory Natalia Sinaga, Rahmat Tubagus Hakiem; https://doi.org/10.1088/1755-1315/366/1/012034 (research study in Indonesia)
5. Low impact development (LID) as an effort to achieve a sustainable urban drainage system (SUDS). Case study: Left side of Garang River Segment VI Semarang - Journal: E3S Web of Conferences year 2019, Authors: Anik Sarminingsih, **Ganjar Samudro,** and Aisyatul Mas'adah**;** https://doi.org/10.1051/e3sconf/20191250 (research study in Indonesia)
6. Performance Evaluation of Jatibarang Reservoir Due to Land Use Changing - Journal: E3S Web of Conferences year 2020, Authors: Anik Sarminingsih, and **Ganjar Samudro**; https://doi.org/10.1088/1755-1315/448/1/012009 (research study in Indonesia)
7. Scientific review of the greening approach to minimizing impact of runoff on land surface - Journal: Revista De Educacion year 2020, Authors: Harida Samudro, **Ganjar Samudro**, and Sarwoko Mangkoedihardjo; http://repository.uin-malang.ac.id/5493/ (research study in Indonesia)
8. Crack formation behavior of composite fly ash–bentonite (FAB) in landfill liner system - IOP Conference Series: Earth and Environmental Science year 2021, Authors: Mochammad Arief Budihardjo, Endro Sutrisno, Winardi Dwi Nugraha, and

Ganjar Samudro; https://doi.org/10.1088/1755-1315/894/1/012027 (research study in Indonesia)

9. Assessment of domestic waste management in Demak Regency, Indonesia - IOP Conference Series: Earth and Environmental Science year 2021, Authors: Bimastyaji Surya Ramadan, Winardi Dwi Nugraha, **Ganjar Samudro**, and Rika Ardiana; https://doi.org/10.1088/1755-1315/894/1/012039 (research study in Indonesia)
10. Prevention of indoor air pollution through design and construction certification: A review of the sick building syndrome conditions - Journal: Journal of Air Pollution and Health year 2022, Authors: Harida Samudro, **Ganjar Samudro**, Sarwoko Mangkoedihardjo; https://doi.org/10.18502/japh.v7i1.8922 (research study in Indonesia)
11. Retrospective Study on Indoor Bioaerosol - Prospective Improvements to Architectural Criteria in Building Design - Journal: Israa University Journal of Applied Science year 2022, Authors: Harida Samudro, **Ganjar Samudro**, Sarwoko Mangkoedihardjo; https://doi.org/10.52865/LSBY9811 (research study in Indonesia)
12. Overview of Indoor Plants: Phytoarchitecture as A Building Health Platform – Journal: Journal of Design and Built Environment year 2022, Authors: Harida Samudro, **Ganjar Samudro**, Sarwoko Mangkoedihardjo; https://ejournal.um.edu.my/index.php/jdbe/article/view/40165/15336 (research study in Indonesia)
13. Cleaning up black carbon using plant strategies – Journal: Plant Science Today year 2023, Authors: Harida Samudro, **Ganjar Samudro**, Sarwoko Mangkoedihardjo; https://doi.org/10.14719/pst.2179 (research study in Indonesia)