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DIPLOMA THESIS

Study of modified metal surfaces as electrodes in microbial electrolyzer cells

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ΕΘΝΙΚΟ ΜΕΤΣΟΒΙΟ ΠΟΛΥΤΕΧΝΕΙΟ
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ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Μελέτη τροποποιημένων μεταλλικών επιφανειών ως ηλεκτρόδια σε μικροβιακές κυψελίδες ηλεκτρόλυσης.

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Abstract

The ever-growing concerns about environmental pollution, specifically CO_2 emissions, have led to an increasing need for more sustainable solutions. Hydrogen can offer a solution to the problem of the intermittent nature of renewable energy. In this thesis, a comparatively new hydrogen production method is being investigated, Microbial Electrolytic Cells (MECs). More specifically, because of the important role that the cathode electrode plays in the Hydrogen Evolution Reaction (HER), the main focus of the experiments being conducted is the enhancement of Hydrogen production by modifying the cathode electrodes and assessing their performance.

The experimental procedure is divided into two main sections. The first one is the modification and analysis of the cathodic electrodes and the second is the operation and result analysis of the Microbial Electrolytic Cell. Firstly, the modification of the stainless-steel cathodes was achieved with a sandblasting technique using various particle sizes of aluminum oxide (Al_2O_3), under the same conditions. The surface of the modified stainless-steel cathodes was then studied using surface analysis techniques, specifically Profilometry and Scanning Electron Microscopy (SEM), to characterize the surface modifications. In the second section, the MEC operation and result analysis is where the Hydrogen production occurs and is measured via Gas Chromatography. Moreover, the electrochemical interactions in the reactor are measured via a Potentiostat.

Finally, the results indicate that the highest surface roughness was exhibited by the cathode that was sandblasted with the "medium" sized grain (F120), which was then proved by the SEM imaging where the inconsistencies were much more visible in comparison to the cathodes that were sandblasted with the smallest or the biggest grains of sand. On the other hand, the MEC results indicated that the highest Hydrogen production was achieved when using the cathode that was sandblasted with the smallest grain (F220 cathode), which had fewer inconsistencies than the F120 and more than the cathode that was sandblasted with the biggest grain (F80). This suggests that the F220 cathode exhibits the optimal surface composition and catalytic properties to facilitate superior hydrogen evolution.

What is more, the optimal MEC operation was investigated, and the crucial role of microbial activity and the presence of biofilm on the anode was proved. Simultaneously, it was found that removing sludge from the solution prevented methanogenesis, ensuring a focus on maximizing hydrogen production. Gas chromatography results showed a notable increase in hydrogen production when CO_2 was used as the purge gas. The increased surface area and more active sites resulting from sandblasting facilitated greater microbial activity and more efficient electron transfer, ultimately boosting hydrogen output, in comparison to the non-sandblasted electrodes.

Concluding, some recommendations for further research would be additional surface analysis of the sandblasted cathodes, using X-ray Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM) for dtailed analysis of the cathode surfaces. Lastly, Impedance spectroscopy analysis is also suggested to further understand the resistance encountered in electron transfer.

Key Words: Microbial Electrolysis Cells (MECs), Hydrogen Evolution Reaction (HER), cathode electrode modification, sandblasting techniques, surface roughness, electrochemical analysis, gas chromatography (GC), stainless steel cathodes, biohydrogen production.

Περίληψη

Οι ολοένα αυξανόμενες ανησυχίες για την περιβαλλοντική ρύπανση και συγκεκριμένα για τις εκπομπές CO_2, έχουν οδηγήσει σε μια εντεινόμενη ανάγκη για πιο βιώσιμες λύσεις. Το υδρογόνο μπορεί να προσφέρει μια λύση στο πρόβλημα της διακοπτόμενης φύσης των ανανεώσιμων πηγών ενέργειας. Στην παρούσα διπλωματική, διερευνάται μια συγκριτικά νέα μέθοδος παραγωγής υδρογόνου, τα Μικροβιακά Ηλεκτρολυτικά Κελιά (MECs). Πιο συγκεκριμένα, λόγω του σημαντικού ρόλου που διαδραματίζει το ηλεκτρόδιο της καθόδου στην αντίδραση εξέλιξης του υδρογόνου (Hydrogen Evolution Reaction- HER), μεγαλύτερη έμφαση ανάμεσα στα πειράματα που διεξάγονται, δίνεται στην ενίσχυση της παραγωγής υδρογόνου μέσω της τροποποίησης των ηλεκτροδίων της καθόδου και η αξιολόγηση της απόδοσής τους.

Η πειραματική διαδικασία χωρίζεται σε δύο κύριες ενότητες. Η πρώτη είναι η τροποποίηση και η ανάλυση των καθοδικών ηλεκτροδίων και η δεύτερη είναι η λειτουργία και η ανάλυση των αποτελεσμάτων του μικροβιακού ηλεκτρολυτικού κελιού. Αρχικά, η τροποποίηση των καθόδων από ανοξείδωτο χάλυβα επιτεύχθηκε με τεχνική αμμοβολής χρησιμοποιώντας διάφορα μεγέθη κόκκων (3 διαφορετικά) οξειδίου του αργιλίου (Al_2O_3), υπό τις ίδιες συνθήκες. Στη συνέχεια, η επιφάνεια των τροποποιημένων καθόδων από ανοξείδωτο χάλυβα μελετήθηκε με τη χρήση τεχνικών ανάλυσης επιφανειών, συγκεκριμένα με τη χρήση προφιλομετρίας και ηλεκτρονικής μικροσκοπίας σάρωσης (SEM), για τον χαρακτηρισμό των επιφανειακών τροποποιήσεων.

Στη δεύτερη ενότητα, μελετάται η λειτουργία του MEC, όπου και λαμβάνει χώρα η παραγωγή υδρογόνου, και η αναλύονται τα αποτελέσματα της λειτουργίας του, η παραγωγή του υδρογόνου, μέσω της αέριας χρωματογραφίας. Επιπλέον, οι ηλεκτροχημικές αλληλεπιδράσεις που συμβαίνουν στον αντιδραστήρα μετρώνται μέσω ενός ποτενσιοστάτη.

Τέλος, τα αποτελέσματα δείχνουν ότι η μεγαλύτερη επιφανειακή τραχύτητα παρουσιάστηκε από την κάθοδο που αμμοβολήθηκε με τον "μεσαίου" μεγέθους κόκκο (F120), γεγονός που αποδείχθηκε στη συνέχεια από την απεικόνιση SEM όπου οι ασυνέχειες ήταν πολύ πιο ορατές σε σύγκριση με τις καθόδους που

αμμοβολήθηκαν με τους μικρότερους (F220) ή τους μεγαλύτερους κόκκους άμμου (F80). Από την άλλη πλευρά, τα αποτελέσματα του MEC έδειξαν ότι η υψηλότερη παραγωγή υδρογόνου επιτεύχθηκε όταν χρησιμοποιήθηκε η κάθοδος που αμμοβολήθηκε με τον μικρότερο κόκκο (κάθοδος F220), η οποία είχε λιγότερες ασυνέπειες από την F120 και περισσότερες από την κάθοδο που αμμοβολήθηκε με τον μεγαλύτερο κόκκο (F80). Αυτό υποδηλώνει ότι η κάθοδος F220 παρουσιάζει τη βέλτιστη σύνθεση επιφάνειας και καταλυτικές ιδιότητες για να ευνοεί την παραγωγή υδρογόνου.

Επιπλέον, διερευνήθηκε η βέλτιστη λειτουργία της MEC και αποδείχθηκε ο κρίσιμος ρόλος της μικροβιακής δραστηριότητας και της παρουσίας βιοφίλμ στην άνοδο. Ταυτόχρονα, διαπιστώθηκε ότι η απομάκρυνση της ιλύος από το διάλυμα αποτρέπει τη μεθανογένεση, εξασφαλίζοντας τη μεγιστοποίηση της παραγωγής υδρογόνου. Τα αποτελέσματα της αέριας χρωματογραφίας έδειξαν αξιοσημείωτη αύξηση της παραγωγής υδρογόνου όταν η αντίδραση γινόταν σε περιβάλλον CO_2. Η αυξημένη επιφάνεια (συγκριτικά με την ανεπεξέργαστη επιφάνεια ανοξείδωτου χάλυβα) και οι περισσότερες ενεργές θέσεις που προέκυψαν από την αμμοβολή διευκόλυναν τη μεγαλύτερη μικροβιακή δραστηριότητα και την αποτελεσματικότερη μεταφορά ηλεκτρονίων, ενισχύοντας τελικά την παραγωγή υδρογόνου.

Συμπερασματικά, ορισμένες συστάσεις για περαιτέρω έρευνα θα ήταν η πρόσθετη επιφανειακή ανάλυση των καθόδων, με τη χρήση φασματοσκοπίας φωτοηλεκτρονίων ακτίνων Χ (XPS) και μικροσκοπίας ατομικής δύναμης (AFM) για λεπτομερή ανάλυση των επιφανειών των καθόδων. Τέλος, προτείνεται επίσης η ανάλυση φασματοσκοπίας σύνθετης αντίστασης για την περαιτέρω κατανόηση της αντίστασης που συναντάται κατά τη μεταφορά ηλεκτρονίων.

Λέξεις Κλειδιά: Μικροβιακά Ηλεκτρολυτικά Κελιά (MECs), Αντίδραση Εξέλιξης Υδρογόνου (HER), τροποποίηση καθοδικού ηλεκτροδίου, τεχνικές αμμοβολής, τραχύτητα επιφάνειας, ηλεκτροχημική ανάλυση, αέρια χρωματογραφία (GC), καθοδικά ηλεκτρόδια από ανοξείδωτο χάλυβα, παραγωγή βιοϋδρογόνου.

1. Introduction

1.1 Global Energy Context & Climate Change.

The urgent need for sustainable energy solutions has never been more apparent as the world grapples with the dual challenges of climate change and limited fossil fuel reserves. The combustion of fossil fuels by human activities is the primary source of anthropogenic greenhouse gas emissions, leading to global warming and subsequent climate disruptions. The primary consequences of utilizing traditional fossil fuel resources are heightened global warming and increased emissions of CO_2 [1]. The figure below shows global CO_2 emissions from fossil fuels, divided into emissions from China (red shading), India (yellow), the US (bright blue), EU (dark blue) and the remainder of the world (grey).

[1] [] Pour Azarm, Elham and Verma, Reetu, Sustainable Energy Solution for Climate Change: Combating Co2 Emissions in Iran. Available at SSRN: https://ssrn.com/abstract=4015155 or http://dx.doi.org/10.2139/ssrn.4015155

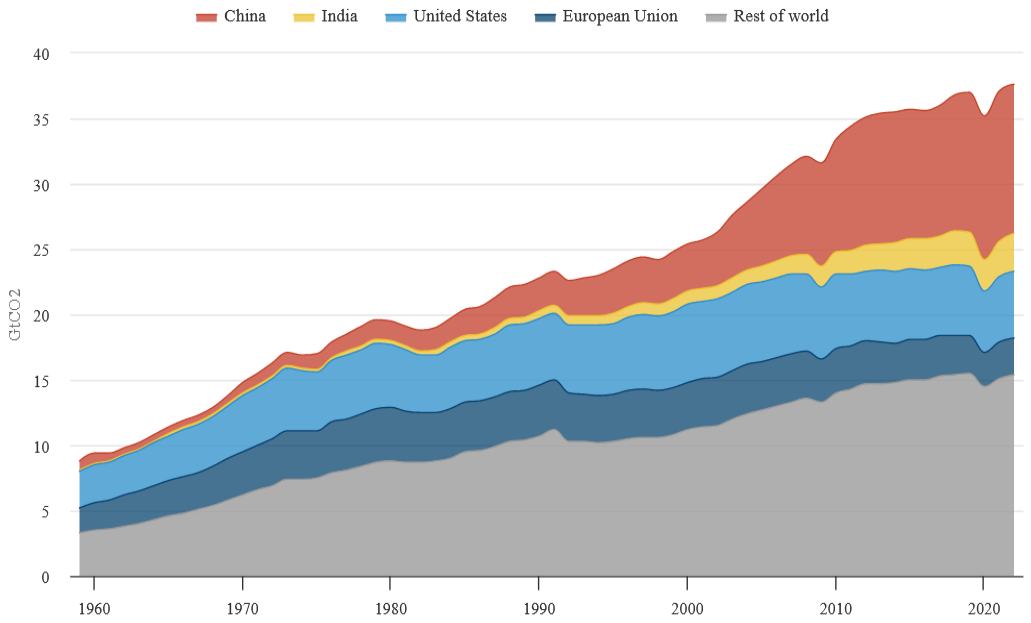

Figure 1: Annual fossil CO_2 emissions for major emitters and rest-of-the-world from 1959-2022. [2]

Coal is the leading contributor to emissions among fossil fuels, accounting for around 40% of global fossil CO_2 emissions as of 2022. Following coal, oil is the second-largest emitter (32%), with gas and cement production contributing 21% and 4%, respectively. These percentages reflect both the global use of each fossil fuel and the differences in CO_2 intensity. Coal emits the most CO_2 per unit of heat or energy generated, followed by oil and gas.

In 2015, the Paris Agreement established a goal to limit the increase in global average surface temperature to below 2°C relative to pre-industrial levels [3]. To achieve this "safe" temperature limit, over 30% of oil, 50% of gas, and 80% of coal reserves must

[2] [] Hausfather, Z. Analysis: Global CO2 Emissions from Fossil Fuels Hit Record High in 2022. Available online:https://www.carbonbrief.org/analysis-global-co2-emissions-from-fossil-fuels-hit-record-high-in-2022/

[3] [] Schleussner, C.-F., Lissner, T.K., Fischer, E.M., Wohland, J., Perrette, Mahé, Golly, A., Rogelj, J. , Childers, K., et al. (2016). *Differential climate impacts for policy-relevant limits to global warming: the case of 1.5°C and 2°C. Earth System Dynamics* 7 327-351. 10.5194/esd-7-327-2016.

remain unexploited by 2050 [4]. Therefore, transitioning away from fossil fuels towards renewable energy sources is imperative to mitigate these emissions and address climate change [5]. According to Holechek et al. renewable energy can replace fossil fuels by 2050, but that requires aggressive application of major lifestyle changes in developed countries, and close cooperation among all countries [6]. Renewable energy sources, such as solar, wind, hydro, geothermal, ocean thermal energy conversion (OTEC), and biomass, are major possibilities for replacing fossil fuels and producing electricity [7]. However, the intermittent nature of renewable energy sources necessitates the implementation of efficient storage mechanisms to mitigate fluctuations, thereby rendering them comparatively less reliable while simultaneously increasing the cost [8].

1.2 Hydrogen as a solution.

Hydrogen can offer a solution to this problem, by harnessing these intermittent energy resources, due to its capacity to function as both a fuel, an energy carrier and storage medium. Hydrogen is an energy vector rather than a fundamental energy source, which means that it must be produced through a chemical process that determines if hydrogen will be deemed renewable, meaning that it is only

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[4][] McGlade, C., Ekins, P. The geographical distribution of fossil fuels unused when limiting global warming to 2 °C. *Nature* **517**, 187–190 (2015). https://doi.org/10.1038/nature14016

[5][] Fonseca, J. D., Camargo, M., Commenge, J., Laurent, F., & Gil, I. D. (2019). Trends in design of distributed energy systems using hydrogen as energy vector: A systematic literature review. *International Journal of Hydrogen Energy*, *44*(19), 9486–9504. https://doi.org/10.1016/j.ijhydene.2018.09.177

[6][] Holechek, J., Geli, H., Sawalhah, M., & Valdez, R. (2022). A Global Assessment: Can Renewable Energy Replace Fossil Fuels by 2050?. Sustainability. https://doi.org/10.3390/su14084792.

[7][] Ishaq, H.; Dincer, I. The Role of Hydrogen in Global Transition to 100% Renewable Energy. In Lecture Notes in Energy; Springer International Publishing: Cham, Switzerland, 2020; pp. 275–307. ISBN 9783030407377.

[8][] *Hydrogen production from water Electrolysis: Current status and future trends*. (2012, February 1). IEEE Journals & Magazine | IEEE Xplore. https://ieeexplore.ieee.org/document/5898382

considered renewable if the energy used to drive the chemical reaction to obtain it is also renewable.

Hydrogen is a promising alternative with numerous advantages over fossil fuels. It is environmentally benign, colorless, tasteless, odorless, lightweight, and non-toxic [[9]]. When used as fuel, the only combustion byproduct is water. This carbon-neutral attribute in conjunction with the fact that hydrogen has a larger calorific value (120-142 MJ/kg) than most alternatives, including CH_4 (50 MJ/kg) and ethanol (26.8 MJ/kg) [[10]] has positioned it as an emerging energy solution and potential fuel source on a global scale, garnering academic attention and interest.Additionally, the infrastructure for fuel storage and transportation that is now used for other chemical fuels is being assessed as a viable alternative for hydrogen storage and distribution which is important because it reduces the need for extensive new investments, accelerates the transition to cleaner energy, and leverages current distribution networks for a smoother and more cost-effective adoption.

Despite being the most abundant element on Earth, hydrogen cannot be found alone in nature and thus it has to be generated. Hydrogen production methods are commonly classified into three primary categories: Green Hydrogen, Grey Hydrogen, and Blue Hydrogen. Green Hydrogen is derived from renewable energy sources such as solar and wind power, predominantly through water electrolysis, resulting in negligible direct carbon dioxide emissions. Grey Hydrogen, conversely, is typically derived from fossil fuels like natural gas or coal, with coal gasification being a prevalent technique [[11]]. Blue Hydrogen, also sourced from fossil fuels,

[9][] Aboelela, D., & Soliman, M. (2022). Hydrogen production from microbial electrolysis cells powered with microbial fuel cells. *Journal of King Saud University. Engineering Sciences/Mağallaẗ Ğāmi'aẗ Al-malik Sa'ūd. al-'Ulūm Al-handsiyyaẗ*. https://doi.org/10.1016/j.jksues.2022.05.008

[10][] Kadier, A., Kalil, M. S., Abdeshahian, P., Chandrasekhar, K., Mohamed, A., Azman, N. F., Logroño, W., Simayi, Y., & Hamid, A. A. (2016). Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals. *Renewable & Sustainable Energy Reviews*, *61*, 501–525. https://doi.org/10.1016/j.rser.2016.04.017

[11][] R. Yukesh Kannah, S. Kavitha, Preethi, O. Parthiba Karthikeyan, Gopalakrishnan Kumar, N. Vo. Dai-Viet, J. Rajesh Banu, Techno-economic assessment of various hydrogen production methods – A review, Bioresource

employs carbon capture and storage (CCS) technologies to mitigate carbon emissions, typically through coal gasification or natural gas processes [12]. Notably, Green Hydrogen emerges as the most environmentally benign alternative owing to its utilization of renewable energy sources and its minimal greenhouse gas emissions impact. This method offers a sustainable and clean way to produce hydrogen without contributing to greenhouse gas emissions.

Hydrogen production methods include electrolysis using renewable energy, thermo-chemical and hybrid processes [13], direct air electrolysis [14], steam reforming of natural gas [15 16], and biological methods like dark fermentation and microbial electrolysis [17].

At present, electrolysis stands as a promising method for extracting hydrogen from water. Water electrolysis has the potential to convert electrical energy to chemical energy, which can then be stored, transferred, and consumed or converted back into

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Technology, Volume 319, 2021, 124175, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2020.124175.

[12] Norazlianie Sazali, Emerging technologies by hydrogen: A review, International Journal of Hydrogen Energy, Volume 45, Issue 38, 2020, Pages 18753-18771, ISSN 0360-3199, https://doi.org/10.1016/j.ijhydene.2020.05.021.

[13] Şahin, S., & Şahin, H. (2021). Generation-IV reactors and nuclear hydrogen production. *International Journal of Hydrogen Energy*. https://doi.org/10.1016/J.IJHYDENE.2020.12.182.

[14] Guo, J., Zhang, Y., Zavabeti, A., Chen, K., Guo, Y., Hu, G., Fan, X., & Li, G. (2021). Hydrogen production from the air. *Nature Communications*, 13. https://doi.org/10.1038/s41467-022-32652-y.

[15] Benghanem, M., Mellit, A., Almohamadi, H., Haddad, S., Chettibi, N., Alanazi, A., Dasalla, D., & Alzahrani, A. (2023). Hydrogen Production Methods Based on Solar and Wind Energy: A Review. *Energies*. https://doi.org/10.3390/en16020757.

[16] Kannah, R., Kavitha, S., , P., Karthikeyan, O., Kumar, G., Dai-Viet, N., & Banu, J. (2020). Techno-economic assessment of various hydrogen production methods - A review.. *Bioresource technology*, 319, 124175 . https://doi.org/10.1016/j.biortech.2020.124175.

[17] Aydin, M., Karaca, A., Qureshy, A., & Dincer, I. (2020). A comparative review on clean hydrogen production from wastewaters.. *Journal of environmental management*, 279, 111793 . https://doi.org/10.1016/j.jenvman.2020.111793.

electricity as needed [18 19]. From an economic standpoint, challenges persist in the electrochemical process. The integration of these technologies into large-scale, economically viable systems necessitates further advancements, as electrolysis demands a substantial amount of electricity for the dissociation of water into hydrogen and oxygen. Moreover, the cost associated with electrolysis equipment and the electricity required for operation can be considerable, potentially hindering its economic competitiveness when compared to conventional hydrogen production techniques [20 21]. To specify, the cost of producing hydrogen from methane reforming in Europe can be between two to five times lower than the cost of electrolysis due to the high electricity consumption [22]. Additionally, the durability of electrolysis equipment, particularly the membranes and electrodes, poses a challenge, impacting the long-term viability and maintenance costs of the technology. Finally, the reliance on electricity, especially if derived from non-renewable sources, can compromise the overall efficiency and environmental sustainability of the process. Consequently, water splitting electrolysis is a promising pathway for efficient hydrogen production, but requires active, stable, and low-cost catalysts for practical use [23].

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[18] Kai Zeng, Dongke Zhang, Recent progress in alkaline water electrolysis for hydrogen production and applications, Progress in Energy and Combustion Science, Volume 36, Issue 3, 2010, Pages 307-326, ISSN 0360-1285, https://doi.org/10.1016/j.pecs.2009.11.002.

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[22] Astiaso Garcia, D.; Barbanera, F.; Cumo, F.; Di Matteo, U.; Nastasi, B. Expert Opinion Analysis on Renewable Hydrogen Storage Systems Potential in Europe. Energies 2016, 9, 963. https://doi.org/10.3390/en9110963

[23] Wang, S., Lu, A., & Zhong, C. (2021). Hydrogen production from water electrolysis: role of catalysts. *Nano Convergence*, 8. https://doi.org/10.1186/s40580-021-00254-x.

1.3 Microbial Electrolysis Cells.

Biological processes present a sustainable and efficient method for hydrogen production, operating under ambient conditions of temperature and pressure and demanding minimal energy input. Bioelectrochemical systems (BESs) in particular, an emerging bioenergy technology, hold significant potential for simultaneously treating wastewater and generating electric energy or producing valuable chemicals [24 25 26]. These systems (BESs) can be categorized based on their electricity utilization. The first type, microbial fuel cells (MFCs), generate electricity from organic waste streams. The second type, microbial electrolysis cells (MECs), require an enforcement of voltage difference to produce hydrogen from organic waste streams [27].

In the early stages of bioelectrochemical systems research, microbial fuel cells (MFCs) were the primary focus. While MFCs are interesting, researchers have recognized that the economic and environmental benefits of electricity generated from MFCs are not yet competitive with other energy sources. Meanwhile, hydrogen production is garnering increasing attention. Consequently, recent developments have aimed to expand the scope of MFCs towards more value-added applications, including hydrogen production through microbial electrolysis cells (MECs).

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[24] [] Yifeng Zhang, Irini Angelidaki, Microbial electrolysis cells turning to be versatile technology: Recent advances and future challenges, Water Research, Volume 56, 2014, Pages 11-25, ISSN 0043-1354, https://doi.org/10.1016/j.watres.2014.02.031.

[25] [] Zhang, Y., & Angelidaki, I. (2014). Microbial electrolysis cells turning to be versatile technology: Recent advances and future challenges. *Water Research*, *56*, 11–25. https://doi.org/10.1016/j.watres.2014.02.031

[26] [] Jacobson, K. S., Drew, D. M., & He, Z. (2011). Efficient salt removal in a continuously operated upflow microbial desalination cell with an air cathode. *Bioresource Technology*, *102*(1), 376–380. https://doi.org/10.1016/j.biortech.2010.06.030

[27] [] Kundu, A., Sahu, J. N., Redzwan, G., & Hashim, M. (2013). An overview of cathode material and catalysts suitable for generating hydrogen in microbial electrolysis cell. *International Journal of Hydrogen Energy*, *38*(4), 1745–1757. https://doi.org/10.1016/j.ijhydene.2012.11.031

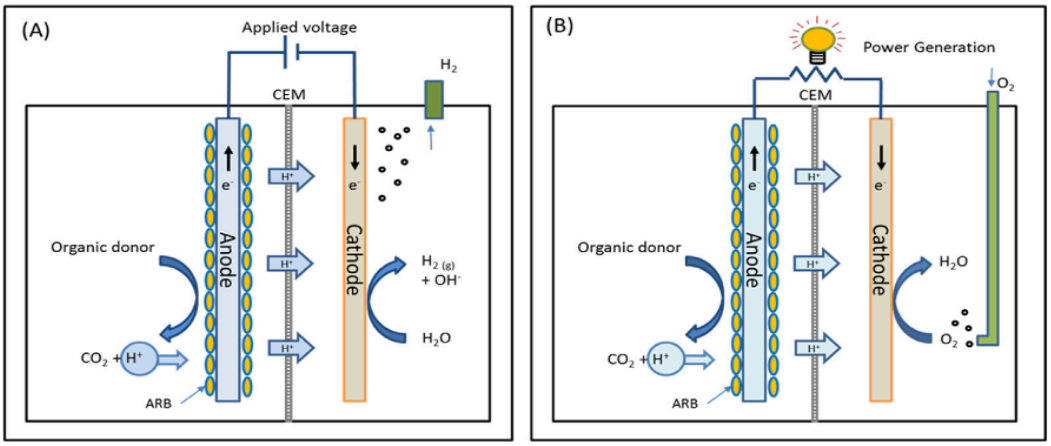

Figure 2: Schematic of (A) MEC and (B) MFC systems consisting of two-compartment cells of anolyte and catholyte separated by cation exchange membrane (CEM). MEC produces H 2 by combining substrate utilization reaction at the anode by ARBs under small, applied potential and MFC produces electric current by combining two half-cell reactions of substrate utilization at the anode and oxygen reduction at the cathode. [28]

In bio-electrochemical systems, bacteria oxidize organic matter, releasing carbon dioxide and protons into the solution, while electrons are transferred to the anode. These electrons travel through an external electrical circuit to the cathode, where they participate in the reduction of oxygen. When oxygen is provided to the cathode, the system, known as a microbial fuel cell (MFC), generates current. Conversely, in the absence of oxygen and by electrochemically enhancing the cathode potential within an MFC circuit, hydrogen can be directly produced through the reduction of protons and electrons generated by the bacteria [29 30]. Comparatively to abiotic water electrolysis, the principal advantage of microbial electrolysis cells (MECs) is that the oxidation process involves organic compounds rather than water, which can take

[28] [] Yasri, N., Roberts, E. P., & Gunasekaran, S. (2019). The electrochemical perspective of bioelectrocatalytic activities in microbial electrolysis and microbial fuel cells. Energy Reports, 5, 1116–1136. https://doi.org/10.1016/j.egyr.2019.08.007

[29] [] Wrana, N., Sparling, R., Cicek, N., & Levin, D. B. (2010). Hydrogen gas production in a microbial electrolysis cell by electrohydrogenesis. *Journal of Cleaner Production*, *18*, S105–S111. https://doi.org/10.1016/j.jclepro.2010.06.018

[30] [] Rozendal, R., Hamelers, H., Euverink, G., Metz, S., & Buisman, C. (2006). Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *International Journal of Hydrogen Energy*, *31*(12), 1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

place at considerably lower redox potentials. As a result, the thermodynamic cell voltage of an MEC is substantially lower than the established 1.23 V threshold for water electrolysis under standard conditions [31 32].

Additionally, another advantage of MECs over other biological hydrogen generation techniques, is that they can utilize a variety of organic materials, including cellulose, glucose, glycerol, acetic acid, sewage sludge, and other wastewaters [33 34] while the byproducts of dark fermentation (e.g., acetate) can also be utilized to produce hydrogen [35].

2. Thesis Scope.

The aim of this thesis is to study the cathode electrode and its material (stainless-steel), focusing mainly on physical alterations on their surfaces, achieved by using sandblasting techniques. Sandblasting is a relatively simple, cost-effective, and scalable technique, making it an attractive option for enhancing electrode performance in practical applications. Through surface modification of the cathodes, the possibility of hydrogen production amplification is explored. The enhanced surface area due to sandblasting is expected to lead to greater and more efficient electron transfer, ultimately increasing hydrogen (H_2) output in MECs. By studying and thoroughly examining the effects of sandblasting on stainless-steel cathodes, through gas and electrochemical analysis this research aims to gather insights into

[31] [] Rozendal RA, Hamelers HVM, Euverink GJW, Metz SJ, Buisman CJN. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int J Hydrog Energy 2006;31:1632–40. https://doi.org/10.1016/j.ijhydene.2005.12.006.

[32]

[33] [] Cotterill, S. E., Dolfing, J., Curtis, T. P., & Heidrich, E. S. (2018). Community assembly in Wastewater-Fed Pilot-Scale microbial electrolysis cells. *Frontiers in Energy Research*, *6*. https://doi.org/10.3389/fenrg.2018.00098

[34] [] Aiken, D. C., Curtis, T. P., & Heidrich, E. S. (2019b). Avenues to the financial viability of microbial electrolysis cells [MEC] for domestic wastewater treatment and hydrogen production. *International Journal of Hydrogen Energy*, *44*(5), 2426–2434. https://doi.org/10.1016/j.ijhydene.2018.12.029

[35] [] Zhang, Y., & Angelidaki, I. (2014b). Microbial electrolysis cells turning to be versatile technology: Recent advances and future challenges. *Water Research*, *56*, 11–25. https://doi.org/10.1016/j.watres.2014.02.031

how physical modifications can be leveraged to optimize Microbial Electrolytic Cells (MEC) performance. This investigation could lead to more efficient and economically viable methods for hydrogen production, contributing to the advancement of sustainable energy technologies.

3. Literature Review

3.1 Microbial Electrolysis Cell System.

The idea of microbial electrolysis cells (MECs) was initially introduced in 2005 [[36] [37]]. Since then, there has been a growing interest in MECs, as illustrated by the trend shown in the following **Figure 3**, which lead to significant advancements. This increasing attention is fueled by the potential of MECs to provide sustainable solutions for hydrogen production and waste treatment. Early research focused on understanding the basic mechanisms of microbial metabolism and electrochemical interactions. Key milestones in the evolution of Microbial Electrolysis Cells (MECs) include the development of more efficient electrode materials, improvements in reactor design, and the identification of key microbial species involved in the electrohydrogenesis process [[38] [39] [40]].

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[36][] Liu, H., Grot, S., & Logan, B. E. (2005). Electrochemically Assisted Microbial Production of Hydrogen from Acetate. *Environmental Science & Technology*, *39*(11), 4317–4320. https://doi.org/10.1021/es050244p

[37][] Rozendal, R., Hamelers, H., Euverink, G., Metz, S., & Buisman, C. (2006). Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *International Journal of Hydrogen Energy*, *31*(12), 1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

[38][] Slate, A. J., Whitehead, K. A., Brownson, D. A., & Banks, C. E. (2019). Microbial fuel cells: An overview of current technology. *Renewable & Sustainable Energy Reviews*, *101*, 60–81. https://doi.org/10.1016/j.rser.2018.09.044

[39][] Li, M., Zhou, M., Tian, X., Tan, C., McDaniel, C. T., Hassett, D. J., & Gu, T. (2018). Microbial fuel cell (MFC) power performance improvement through enhanced microbial electrogenicity. *Biotechnology Advances*, *36*(4), 1316–1327. https://doi.org/10.1016/j.biotechadv.2018.04.010

[40][] Murugaiyan, J., Narayanan, A., & Mohamed, S. (2022). An overview of microbial electrolysis cell configuration: Challenges and prospects on biohydrogen production. *International Journal of Energy Research*, 46, 20811 - 20827. https://doi.org/10.1002/er.8494.

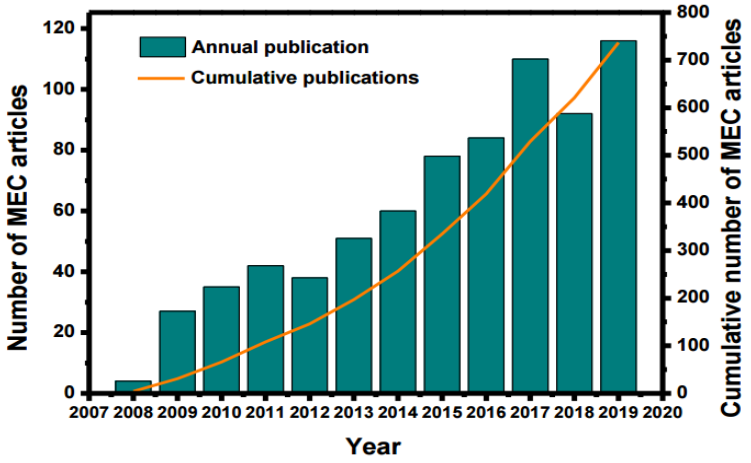

Figure 3: The annual and cumulative numbers of research articles on MECs indexed in WoS from 2008 until 2019.

Despite this interest and the promising advancements in research, the commercialization of MECs is far from being implemented, as shown in **Figure 4**. The primary obstacles to commercial adoption are the high costs, which are largely attributed to the use of expensive materials and the significant maintenance requirements [[41]].

[41][] Escapa, A., San-Martín, M., Mateos, R., & Morán, A. (2015). Scaling-up of membraneless microbial electrolysis cells (MECs) for domestic wastewater treatment: Bottlenecks and limitations. *Bioresource Technology*, *180*, 72–78. https://doi.org/10.1016/j.biortech.2014.12.096

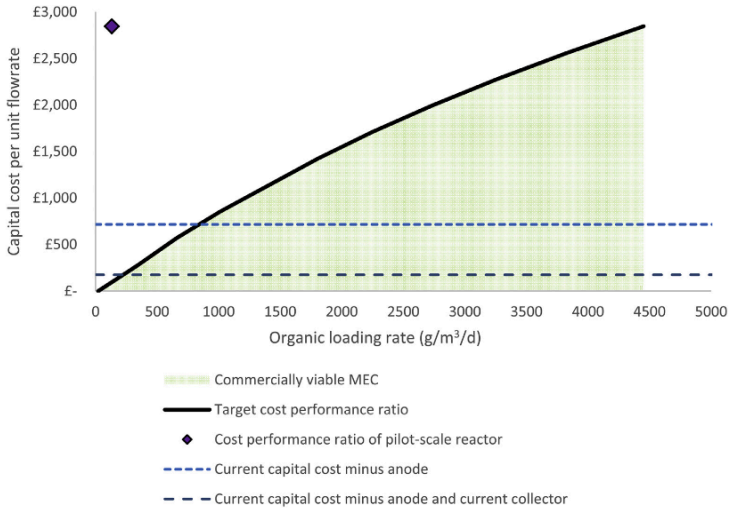

Figure 4: Target cost-performance ratios for financially competitive MECs treating domestic wastewater [[42]].

The cathode is a crucial component of the MEC system. The cathode, including its catalyst, can account for nearly 47% of the total cost of the MEC system, making it a significant factor in the overall expense [[43]]. In order to address these economic challenges for MECs to be considered a viable option and a profitable large-scale operation, further research must be conducted, and more innovative ideas on the materials used have to be suggested.

Understanding the fundamental principles that regulate the behavior of MECs is critical for maximizing their performance and fulfilling their potential across varied applications. MECs are composed of an anodic and a cathodic electrode, which in the case of a dual-chamber are separated by an ion-conducting membrane, resulting in an organized environment where microbial activity leads to electrochemical

[42][] Daniel C. Aiken, Thomas P. Curtis, Elizabeth S. Heidrich, Avenues to the financial viability of microbial electrolysis cells [MEC] for domestic wastewater treatment and hydrogen production, International Journal of Hydrogen Energy, Volume 44, Issue 5, 2019, Pages 2426-2434, ISSN 0360-3199, https://doi.org/10.1016/j.ijhydene.2018.12.029.

[43][] Rozendal RA, Hamelers HVM, Rabaey K, Keller J, Buisman CJN. Towards practical implementation of bioelectrochemical wastewater treatment. Trends Biotechnol 2008;26:450e9.

processes. In the case of a single chambered reactor as featured in **Figure 5**, the anode and cathode electrodes coexist in one compartment, and are submersed in the same solution, which is to be treated by the MEC.

The anodic electrode hosts the microbes, which form a biofilm, and thus is the site of microbial oxidation processes, in which exoelectrogens catalyze the breakdown of organic molecules, producing protons, electrons, and carbon dioxide CO_2 as metabolic byproducts. Electrons released during this process are shuttled to the anode surface, through a circuit under applied voltage.

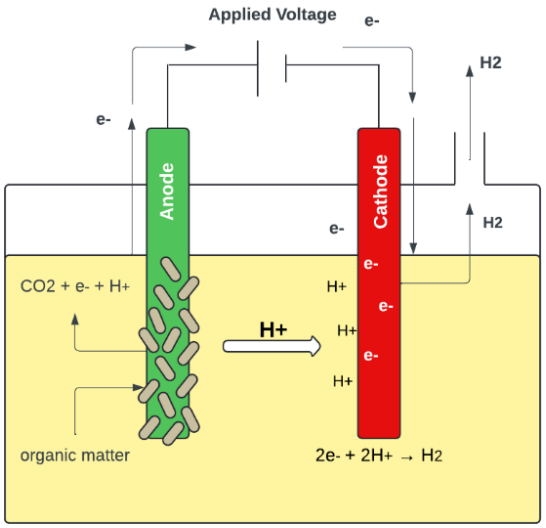

Figure 5: Operational principle of a single chamber microbial electrolysis cell (MEC).

At the cathode, electrons from the anode are spent in reduction processes through forced reduction of protons in anaerobic environments. In the absence of oxygen, an external voltage is used to promote proton reduction, which results in hydrogen gas. In the absence of oxygen, current generation does not occur spontaneously. Under such anaerobic conditions, current can be generated by applying a low voltage

(usually between 0.1 and 0.5 V) between the anode and cathode [44]. This imposed current reduces protons at the cathode, producing hydrogen. This method is known as electrohydrogenesis or microbial electrolysis.

$$8H^{+} ++ 8e^{-} \rightarrow 4H_{2} \quad \textbf{(1)}$$

To produce H_2 at the cathode of MEC from the combination of these protons and electrons, a cathode potential of at least >–0.414 V vs NHE (normal hydrogen electrode) is needed under ordinary biological circumstances of pH=7, T=25°C, and P=1 atm. [45] [46]

For electrode reactions in MECs at a pH of 7, the theoretical reduction potential of each half cell reaction is written and calculated based on the Nernst equation. For the cathode reaction, the theoretical reduction potential is written and calculated according to the following equation:

$$\mathrm{E}_{cat} = E^{o}_{cat} - \frac{RT}{2F} ln \frac{P_{H2}}{\left[H^{+}\right]^{8}} = 0 - \frac{8.314 \times 298.15}{2 \times 96485} ln \frac{1}{\left[10^{-7}\right]^{8}} = -0.414\,V \quad \textbf{(2)}$$

Where E^{o}_{cat} represents the standard electrode potential for hydrogen (0 V), R (8.314 J/K/mol) is the universal gas constant, T (K) is the absolute temperature, and F (96485 C/mol e^{-}) is Faraday's constant.

[44] [] Liu, H., Grot, S., & Logan, B. E. (2005). Electrochemically Assisted Microbial Production of Hydrogen from Acetate. *Environmental Science & Technology*, *39*(11), 4317–4320. https://doi.org/10.1021/es050244p

[45] [] Rozendal, R., Hamelers, H., Euverink, G., Metz, S., & Buisman, C. (2006c). Principle and perspectives of hydrogen production through biocatalyzed electrolysis. International Journal of Hydrogen Energy, 31(12), 1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

[46] [] Sun, M., Mu, Z., Sheng, G., Shen, N., Tong, Z., Wang, H., & Yu, H. (2010). Hydrogen production from propionate in a biocatalyzed system with in-situ utilization of the electricity generated from a microbial fuel cell. International Biodeterioration & Biodegradation, 64(5), 378–382. https://doi.org/10.1016/j.ibiod.2010.04.004

The theoretical reduction potential for the anode reaction, to oxidize 1 g/L (16.9 mM) of acetate, in neutral conditions similar to the inoculum found widely in MFC and MEC systems (i.e. pH = 7 in 5 mM bicarbonate) is written and calculated via the Nernst equation as follows [45] [47]:

$$CH_3COO + H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad \textbf{(3)}$$

$$_{node} = E^o_{anode} - \frac{RT}{24F} ln \frac{[C6H1106^-]}{[H^+]^{24}} = 0,187 - \frac{8,314 \times 298,15}{24 \times 96485} ln \frac{0,169}{(0.05)^2(10^{-7})^9} = -0,300\ V \quad \textbf{(4)}$$

Where E° (0,187 V) is the standard electrode potential for acetate oxidation [48]. Thus, the cell voltage (E_{cell}) necessary for a MEC to produce H_2 at the cathode under these conditions is

$$-0.414V - (-0.300) = -0.114V \qquad (SEQ\ Equation \setminus *\ ARABIC\ 5)$$

The previous equation indicates that the E_{cell} is negative, demonstrating that hydrogen (H_2) cannot be spontaneously produced from acetate in an MEC. To make the reaction favorable and enable hydrogen production, an additional input voltage of at least 0.114V must be supplied. In practice, the applied voltage (E_{ap}) is typically higher than the theoretical E_{cell} due to losses such as ohmic loss, activation loss, and mass transport loss within the MEC system. Previous studies on microbial

[47][] Selembo, P. A., Perez, J. M., Lloyd, W. A., & Logan, B. E. (2009). High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. International Journal of Hydrogen Energy, 34(13), 5373–5381. https://doi.org/10.1016/j.ijhydene.2009.05.002

[48][] Tian, Z., Da, Y., Wang, M., Dou, X., Cui, X., Chen, J., Jiang, R., Xi, S., Cui, B., Luo, Y., Yang, H., Long, Y., Xiao, Y., & Chen, W. (2023). Selective photoelectrochemical oxidation of glucose to glucaric acid by single atom Pt decorated defective TiO2. *Nature Communications*, *14*(1). https://doi.org/10.1038/s41467-023-35875-9

electrolysis cells (MECs) have shown that an E_{ap} of 0.2 V or more is necessary to achieve measurable current and hydrogen production in MECs [49].

Numerous facets of Microbial Electrolysis Cells (MECs) have been examined to boost hydrogen output and enhance wastewater treatment. Research indicates that MECs can be optimized for increased hydrogen generation through a variety of strategies, including the adjustment of operational parameters (such as temperature, solution conductivity and pH [50 51]) the improvement of biofilm and catalyst properties [52]

[49] [] Rozendal, R., Hamelers, H., Euverink, G., Metz, S., & Buisman, C. (2006d). Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *International Journal of Hydrogen Energy*, *31*(12), 1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

[50] [] Fathy, A., Rezk, H., Yousri, D., Alharbi, A., Alshammari, S., & Hassan, Y. (2023). Maximizing Bio-Hydrogen Production from an Innovative Microbial Electrolysis Cell Using Artificial Intelligence. *Sustainability*. https://doi.org/10.3390/su15043730.

[51] [] Guo, K., Prévoteau, A., & Rabaey, K. (2017). A novel tubular microbial electrolysis cell for high rate hydrogen production. *Journal of Power Sources*, 356, 484-490. https://doi.org/10.1016/J.JPOWSOUR.2017.03.029.

[52] [] Wang, L., Long, F., Liang, D., Xiao, X., & Liu, H. (2020). Hydrogen production from lignocellulosic hydrolysate in an up-scaled microbial electrolysis cell with stacked bio-electrodes.. *Bioresource technology*, 320 Pt A, 124314 . https://doi.org/10.1016/j.biortech.2020.124314.

[53], and the refinement of reactor design and control mechanisms [54 55 56] and design choices like electrode and membrane materials [57].

Particularly, there has been extensive research on the cathode electrode material, due to its pivotal role in facilitating the hydrogen evolution reaction. Understanding the properties and characteristics of cathode materials is essential for optimizing MEC performance and enhancing hydrogen production efficiency. Through a comprehensive review of existing literature, this chapter aims to explore the diverse range of cathode materials utilized in MECs, their associated advantages and limitations, and the impact of material properties on overall system performance.

3.2 Cathodic Electrode Materials.

As mentioned earlier, the cathode, including its catalyst, can contribute nearly 47% to the total cost of the MEC system, making it a significant factor in overall expenses. The hydrogen evolution reaction (HER) takes place at the cathode electrode, highlighting its critical importance in the system's functionality. Due to this, there has been a concerted effort by research teams across the globe to extensively study and innovate cathode materials. Their goal is to find the most economical,

[53][] Zhao, N., Liang, D., Meng, S., & Li, X. (2020). Bibliometric and content analysis on emerging technologies of hydrogen production using microbial electrolysis cells. *International Journal of Hydrogen Energy*, 45, 33310-33324. https://doi.org/10.1016/J.IJHYDENE.2020.09.104.

[54][] Lim, S., Fontmorin, J., Salehmin, M., Feng, Y., Scott, K., & Yu, E. (2021). Enhancing hydrogen production through anode fed-batch mode and controlled cell voltage in a microbial electrolysis cell fully catalysed by microorganisms.. *Chemosphere*, 132548 . https://doi.org/10.1016/j.chemosphere.2021.132548.

[55][] Rousseau, R., Etcheverry, L., Roubaud, E., Basséguy, R., Délia, M., & Bergel, A. (2020). Microbial electrolysis cell (MEC): Strengths, weaknesses and research needs from electrochemical engineering standpoint. *Applied Energy*. https://doi.org/10.1016/j.apenergy.2019.113938.

[56][] Miller, A., Singh, L., Wang, L., & Liu, H. (2019). Linking internal resistance with design and operation decisions in microbial electrolysis cells. *Environment International*, *126*, 611–618. https://doi.org/10.1016/j.envint.2019.02.056

[57][] Logan, B. E., Zikmund, E., Yang, W., Rossi, R., Kim, K., Saikaly, P. E., & Zhang, F. (2018). Impact of Ohmic resistance on measured electrode potentials and maximum power production in microbial fuel cells. *Environmental Science & Technology*, *52*(15), 8977–8985. https://doi.org/10.1021/acs.est.8b02055

sustainable, efficient, and durable materials that can enhance the HER. Success in this area could significantly advance the commercialization of MEC systems, making them more viable and widely accessible. This research is not only crucial for reducing costs but also for improving the performance and longevity of the MEC systems, thereby paving the way for a more sustainable energy future.

3.2.1 Carbon Based Cathodes.

Carbon-based cathodes are commonly employed in microbial electrolysis cells (MECs) because of their low cost, high conductivity, and significant surface area. Fe-doped carbon nanofiber cathodes outperform platinum-doped carbon cloth at a lower cost, according to recent research [58]. Carbon-based materials, such graphite felt, have advantages but also confront issues such as lower repeatability and increased mass transfer losses. Furthermore, biofouling and long-term degradation may impact their effectiveness and stability over long periods of time [59].

3.2.2 Platinum Cathodes.

Platinum is frequently incorporated into other substrates or supports rather than being utilized independently as a cathode material. This practice aims to leverage the remarkable catalytic properties of platinum, which have been extensively studied

[58] [] Nandy, A., Farkas, D., Pepió-Tárrega, B., Martinez-Crespiera, S., Borràs, E., Avignone-Rossa, C., & Di Lorenzo, M. (2023). Influence of carbon-based cathodes on biofilm composition and electrochemical performance in soil microbial fuel cells. *Environmental Science & Ecotechnology*, *16*, 100276. https://doi.org/10.1016/j.ese.2023.100276

[59] [] Cario, B., Rossi, R., Kim, K., & Logan, B. (2019). Applying the electrode potential slope method as a tool to quantitatively evaluate the performance of individual microbial electrolysis cell components.. *Bioresource technology*, 287, 121418 . https://doi.org/10.1016/j.biortech.2019.121418.

in the context of microbial fuel cell (MFC) research [60 61 62], while simultaneously avoiding the problem of high cost that comes with the use of Platinum in any application. However, platinum cathode materials in microbial electrolysis cells is often constrained not only by their high cost, but also due to the fact that Pt is prone to poisoning by the buffer in the electrolyte thus rendering them even more economically impractical for widespread use. [63 64].

3.2.3 Nickel Based Cathodes.

Nickel-based electrodes have received considerable attention as potential cathode catalysts. One major issue is their high cost and over-potential loss, which can restrict efficiency levels, thus making them less attractive compared to alternatives like stainless steel [65 66]. A notable challenge in their practical application is the

[60][] Rozendal, R., Hamelers, H., Euverink, G., Metz, S., & Buisman, C. (2006b). Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *International Journal of Hydrogen Energy*, *31*(12), 1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

[61][] Zerrouki, A., Kameche, M., Amer, A., Tayeb, A., Moussaoui, D., & Innocent, C. (2020). Platinum nanoparticles embedded into polyaniline on carbon cloth: improvement of oxygen reduction at cathode of microbial fuel cell used for conversion of medicinal plant wastes into bio-energy. *Environmental Technology*, 43, 1359 - 1369. https://doi.org/10.1080/09593330.2020.1829088.

[62][] Suharto, T., Satar, I., Daud, W., Somalu, M., & Hong, K. (2022). Recent Advancement of Nickel Based-Cathode for The Microbial Electrolysis Cell (MEC) and Its Future Prospect. *Journal of Engineering Science and Technology Review*. https://doi.org/10.25103/jestr.151.24.

[63][] Hu, H., Fan, Y., & Liu, H. (2009). Hydrogen production in single-chamber tubular microbial electrolysis cells using non-precious-metal catalysts. *International Journal of Hydrogen Energy*, 34, 8535-8542. https://doi.org/10.1016/J.IJHYDENE.2009.08.011.

[64][] Suharto, T., Satar, I., Daud, W., Somalu, M., & Hong, K. (2022). Recent Advancement of Nickel Based-Cathode for The Microbial Electrolysis Cell (MEC) and Its Future Prospect. *Journal of Engineering Science and Technology Review*. https://doi.org/10.25103/jestr.151.24.

[65][] Kundu, A., Sahu, J. N., Redzwan, G., & Hashim, M. A. (2013). An overview of cathode material and catalysts suitable for generating hydrogen in microbial electrolysis cell. *International Journal of Hydrogen Energy*, *38*(4), 1745–1757.

[66][] Chaurasia, A. K., & Mondal, P. (2022). Enhancing biohydrogen production from sugar industry wastewater using Ni, Ni–Co and Ni–Co–P electrodeposits as

formation of passivation layers of γ-$Ni(OH)_2$ on their surfaces, which then in turn affects their performance stability [67]. Additionally, nickel electrodes can suffer from reduced long-term durability due to corrosion and fouling in wastewater environments, further limiting their practical application [68 69].

Nevertheless, despite these challenges, numerous research groups persist in exploring the viability of nickel electrodes in MECs for facilitating the hydrogen evolution reaction (HER) and have yielded promising results. In a study by Lu et al., nickel-based catalysts exhibited significant efficiency and stability in generating hydrogen from wastewater, suggesting a promising avenue for cost-effective hydrogen production [70]. Similarly, Jeremiasse et al. used Ni foam as cathode for generating high purity hydrogen at high volumetric production rate [71].

In conclusion, while nickel electrodes exhibit potential for HER enhancement in MECs, challenges persist, particularly concerning performance stability due to

cathodes in microbial electrolysis cells. Chemosphere, 286, 131728. https://doi.org/10.1016/j.chemosphere.2021.131728

[67] Baek, G., Rossi, R., & Logan, B. E. (2021). Changes in electrode resistances and limiting currents as a function of microbial electrolysis cell reactor configurations. Electrochimica Acta, 388, 138590. https://doi.org/10.1016/j.electacta.2021.138590

[68] Choi, C., & Cui, Y. (2012). Recovery of silver from wastewater coupled with power generation using a microbial fuel cell. Bioresource Technology, 107, 522–525. https://doi.org/10.1016/j.biortech.2011.12.058

[69] Hu, H., Fan, Y., & Liu, H. (2008). Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Research, 42(15), 4172–4178. https://doi.org/10.1016/j.watres.2008.06.015

[70] Lu, L., Hou, D., Fang, Y., Huang, Y., & Ren, Z. J. (2016). Nickel based catalysts for highly efficient H2 evolution from wastewater in microbial electrolysis cells. *Electrochimica Acta*, *206*, 381–387. https://doi.org/10.1016/j.electacta.2016.04.167

[71] Jeremiasse, A. W., Hamelers, H. V., Saakes, M., & Buisman, C. J. (2010). Ni foam cathode enables high volumetric H2 production in a microbial electrolysis cell. *International Journal of Hydrogen Energy*, *35*(23), 12716–12723. https://doi.org/10.1016/j.ijhydene.2010.08.131

γ-Ni(OH)2 formation. This emphasizes the need for continued research into cathode catalysts in practical hydrogen production applications [72].

3.2.4 Stainless Steel Cathodes.

Stainless steel is an attractive cathode material for MECs due to its low cost, good conductivity and mechanical strength. It is also resistant to corrosion, so it is suitable for long-term use in various wastewater conditions, a characteristic that is much needed when designing Microbial Electrolytic Cels, since the cathodes are constantly submerged in wastewater that is to be treated.

Stainless- Steel cathodes have been used in many MEC research articles, in different shapes and forms. For instance, Call D. et al. showed that high surface area stainless steel brush cathodes can produce hydrogen at rates and efficiencies comparable to platinum-catalyzed carbon cloth cathodes, effectively eliminating the need for expensive precious metal catalysts [73]. Moreover, Stainless steel woven mesh cathodes in MECs show smaller resistance, leading to higher current density and better system performance compared to other cathodes [74]. In this line of thinking, Zhang, Y. et al. proved that stainless steel mesh (SSM) cathodes exhibit significant potential for developing low-cost MECs for hydrogen production, benefiting from their high specific surface areas that facilitate commercialization [75].

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[72][] Lu, L., Hou, D., Fang, Y., Huang, Y., & Ren, Z. (2016). Nickel based catalysts for highly efficient H2 evolution from wastewater in microbial electrolysis cells. *Electrochimica Acta*, 206, 381-387. https://doi.org/10.1016/J.ELECTACTA.2016.04.167.

[73][] Call, D., Merrill, M., & Logan, B. (2009). High surface area stainless steel brushes as cathodes in microbial electrolysis cells.. *Environmental science & technology*, 43 6, 2179-83 . https://doi.org/10.1021/ES803074X.

[74][] Zhou, R., Zhou, S., & He, C. (2020). Quantitative evaluation of effects of different cathode materials on performance in Cd(II)-reduced microbial electrolysis cells.. *Bioresource technology*, 307, 123198 . https://doi.org/10.1016/j.biortech.2020.123198.

[75][] Zhang, Y., Merrill, M., & Logan, B. (2010). The use and optimization of stainless steel mesh cathodes in microbial electrolysis cells. *International Journal of Hydrogen Energy*, 35, 12020-12028. https://doi.org/10.1016/J.IJHYDENE.2010.08.064.

Additionally, chemical surface modifications on stainless steel electrodes have been investigated, which lead to significant findings. According to Ghasemi, B. et al., modifications of stainless-steel cathodes, such as the addition of polyaniline and graphene, achieve hydrogen production and COD removal rates that are slightly lower than those of conventional platinum cathodes but with significantly lower fabrication costs [[76]]. Specifically, Ni-Co-P electrodeposits on Stainless Steel 316 cathodes have demonstrated improved corrosion stability and catalytic performance, making them viable for commercial applications [[77]]. In another study, it was found that SSM cathode materials in Cd (II)-reduced MECs had a superior performance, which can be attributed to their smaller resistance and higher current density compared to nickel foam (NF) and carbon cloth (CC) cathodes [[78]]. Another interesting stainless-steel cathode application is that of a MEC system which was employed for effective treatment of textile-dyeing wastewater. The stainless-steel cathode led to enhanced biodegradability and reduction of treatment difficulty [[79]]. In a study conducted by Xie, J. et al. stainless-steel cathodes were found to potentially improve BOD_5/COD efficiency by 30,36% and 22,36% compared to conventional carbon cloth cathodes, thus enhancing wastewater biodegradability and microbial interactions [[80]]. Furthermore, stainless steel cathodes enhance the

[76][] Ghasemi, B., Yaghmaei, S., Abdi, K., Mardanpour, M., & Haddadi, S. (2019). Introducing an affordable catalyst for biohydrogen production in microbial electrolysis cells.. *Journal of bioscience and bioengineering*. https://doi.org/10.1016/j.jbiosc.2019.07.001.

[77][] Chaurasia, A., Goyal, H., & Mondal, P. (2020). Hydrogen gas production with Ni, Ni–Co and Ni–Co–P electrodeposits as potential cathode catalyst by microbial electrolysis cells. *International Journal of Hydrogen Energy*, 45, 18250-18265. https://doi.org/10.1016/J.IJHYDENE.2019.07.175.

[78][] Zhou, R., Zhou, S., & He, C. (2020). Quantitative evaluation of effects of different cathode materials on performance in Cd(II)-reduced microbial electrolysis cells.. *Bioresource technology*, 307, 123198 . https://doi.org/10.1016/j.biortech.2020.123198.

[79][] Xie, J., Zou, X., Chang, Y., Xie, J., Liu, H., Cui, M., Zhang, T., & Chen, C. (2022). The microbial synergy and response mechanisms of hydrolysis-acidification combined microbial electrolysis cell system with stainless-steel cathode for textile-dyeing wastewater treatment.. *The Science of the total environment*, 158912 . https://doi.org/10.2139/ssrn.4174980.

[80][] Xie, J., Zou, X., Chang, Y., Xie, J., Liu, H., Cui, M., Zhang, T., & Chen, C. (2022). The microbial synergy and response mechanisms of hydrolysis-acidification

electrocatalytic properties of biofilms, resulting in longer runtimes and higher efficiency [81].

Overall, stainless steel cathodes allow for low cell voltage, high conductivity, and optimal hydrogen production at a low cost [10]. They offer substantial advantages in terms of performance, scalability, and electron transfer kinetics, making them a strong contender for widespread use in MECs [82].

4. Materials and Methods.

4.1 Materials.

The single-chamber Microbial Electrochemical Cell (MEC) described here consists of a cylindrical structure with a diameter of 12 cm. The anode electrode, made of carbon felt, is positioned diametrically opposite the cathode, which is crafted from stainless steel and coated with manganese (Mn). The setup utilizes a synthetic glucose-based wastewater solution as the operational medium. This configuration provides a platform for exploring microbial electrochemistry in applications such as wastewater treatment and energy generation, with a focus on optimizing electron transfer and reaction efficiency.

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combined microbial electrolysis cell system with stainless-steel cathode for textile-dyeing wastewater treatment.. *The Science of the total environment*, 158912 . https://doi.org/10.2139/ssrn.4174980.

[81] [] Erable, B., Byrne, N., Etcheverry, L., Achouak, W., & Bergel, A. (2017). Single medium microbial fuel cell: Stainless steel and graphite electrode materials select bacterial communities resulting in opposite electrocatalytic activities. *International Journal of Hydrogen Energy*, 42, 26059-26067. https://doi.org/10.1016/J.IJHYDENE.2017.08.178.

[82] [] Mier, A., Olvera-Vargas, H., Mejía-López, M., Longoria, A., Verea, L., Sebastian, P., & Arias, D. (2021). A review of recent advances in electrode materials for emerging bioelectrochemical systems: From biofilm-bearing anodes to specialized cathodes.. *Chemosphere*, 283, 131138 . https://doi.org/10.1016/j.chemosphere.2021.131138.

4.1.1 Cathodic Electrode.

The cathode plays a pivotal role in the function of Microbial Electrolysis Cells (MECs) due to its involvement in hydrogen production. During MEC operation, protons migrate to the cathodic section, and electrons travel externally to the cathode where they combine with protons to produce biohydrogen.

As previously stated, several materials have been evaluated for use as cathodes, including titanium, silver mesh, and nickel foam. Despite extensive exploration, carbonaceous materials have demonstrated a relatively slow hydrogen evolution reaction (HER) [21]. Traditionally, carbon paper coated with a platinum (Pt) catalyst has been employed due to its high efficiency. However, platinum is costly, scarce, and prone to contamination by sulfides and cyanides, which diminishes its practicality for MEC applications.

In this study, stainless steel (SS304) was selected as the cathode material. This choice is supported by the superior corrosion resistance offered by its high chromium and nickel content, making it a viable alternative to platinum. The SS304 stainless steel samples used had dimensions of 3x3x0.2 cm and comprised the following elements:

- Iron (Fe): Primary constituent
- Nickel (Ni): 8-10.5%
- Chromium (Cr): 18-20%
- Other elements: Manganese (Mn), Silicon (Si), Carbon (C).

The high nickel and chromium content of SS304 stainless steel is essential for its excellent corrosion resistance properties, which are critical for the long-term operation of the MEC [[83]].

4.1.2 Anode Electrode.

The anode serves as the site for the growth of the active biofilm, where crucial bio-electrochemical reactions occur:

$$C_6H_{12}O_6 + 6\,H_2O \rightarrow 6\,CO_2 + 24\,H^+ + 24e^- \quad (6)$$

[83][] Sedriks, A. J. (1996). *Corrosion of Stainless Steels*. John Wiley & Sons.

An effective anode material must exhibit high conductivity, a large specific surface area and porosity, low cost, biocompatibility, and high stability [84]. Among various carbonaceous materials, such as graphite rods, carbon brushes, graphite felt, plain carbon cloth, and activated carbon, carbon felt has been identified as a promising option due to its high specific surface area, good electrical conductivity, biocompatibility, and ability to enhance electricity generation, substrate fluxes, and bioelectrochemical activity, despite some limitations related to mass transport and internal resistance [85 86 87].

In this line of thinking, carbon cloth was chosen for the anode. The acclimation process involved using an existing Microbial Fuel Cell (MFC) system to foster the growth of exoelectrogens onto the carbon cloth. The acclimation on to the anode electrode was accomplished in another Microbial Fuel Cell, with the following specifics:

Anode Chamber: The anode chamber was inoculated with sludge from an anaerobic digester to promote the growth of an electrochemically active biofilm. The inoculation process involved:

- Utilizing carbon felt as the primary anode material.
- Acclimating exoelectrogens in an existing dual chamber Microbial Fuel Cell onto carbon felt.

[84][] Zhou, M., Chi, M., Luo, J., He, H., & Jin, T. (2013). An overview of electrode materials in microbial fuel cells. *Journal of Power Sources*, 196(10), 4427-4435.

[85][] Zhao, Y., Ma, Y., Li, T., Dong, Z., & Wang, Y. (2018). Modification of carbon felt anodes using double-oxidant HNO3/H2O2 for application in microbial fuel cells. *RSC Advances*, 8, 2059 - 2064. https://doi.org/10.1039/c7ra12923h.

[86][] Hidalgo, D., Tommasi, T., Bocchini, S., Chiolerio, A., Chiodoni, A., Mazzarino, I., & Ruggeri, B. (2016). Surface modification of commercial carbon felt used as anode for Microbial Fuel Cells.. *Energy*, 99, 193-201. https://doi.org/10.1016/J.ENERGY.2016.01.039.

[87][] Santoro, C., Arbizzani, C., Erable, B., & Ieropoulos, I. (2017). Microbial fuel cells: From fundamentals to applications. A review. *Journal of Power Sources*, 356, 225-244. Santoro, C., Arbizzani, C., Erable, B., & Ieropoulos, I. (2017). Microbial fuel cells: From fundamentals to applications. A review. Journal of Power Sources, 356, 225–244. https://doi.org/10.1016/j.jpowsour.2017.03.109

- Employing glucose as the organic substrate, with the biofilm growth occurring under batch operation, continuously stirred with a magnetic stirrer.

Each acclimation cycle lasted 10 days, with a total of 11 batch cycles completed. The volume filling for each cycle was as follows:

- Buffer solution: 87.5 mL (pH 7)
- Glucose solution: 10 mL with a final Chemical Oxygen Demand (COD) of ~1000 mg/L
- Sludge inoculum: 2.5 mL grown with salts

Cathode Chamber: The cathode chamber, also made of carbon felt, was filled with 100 mL of pH 7 buffer solution.

This setup and procedure ensured optimal conditions for biohydrogen production in the MEC reactor, leveraging the properties of the chosen materials and specific configuration to enhance efficiency and performance.

4.1.3 Reactor Configuration.

The Microbial Electrolysis Cell (MEC) reactor setup is comprised of several components to ensure its proper functioning. As seen in Figure 6, the core components are the top and bottom cap and of course the chamber cell. The top cap included two electrode wires (black for the anode and white for the cathode), in order for them t be easily identified and connected during assembly and operation. This cap also featured a hole for a tube to pass through the cell and exit at the bottom cap, as well as a small inlet for adding the synthetic wastewater solution. The bottom cap had an outlet for the treated solution, allowing efficient removal.

The single chamber cell, cylindrical in shape, had an internal diameter of 10 cm and a working volume of 200 mL. The cell was constructed from Ertalon, chosen for its durability and resistance to chemicals within the reactor. This material selection ensured the cell could maintain structural integrity and function effectively in the internal environment of the MEC.

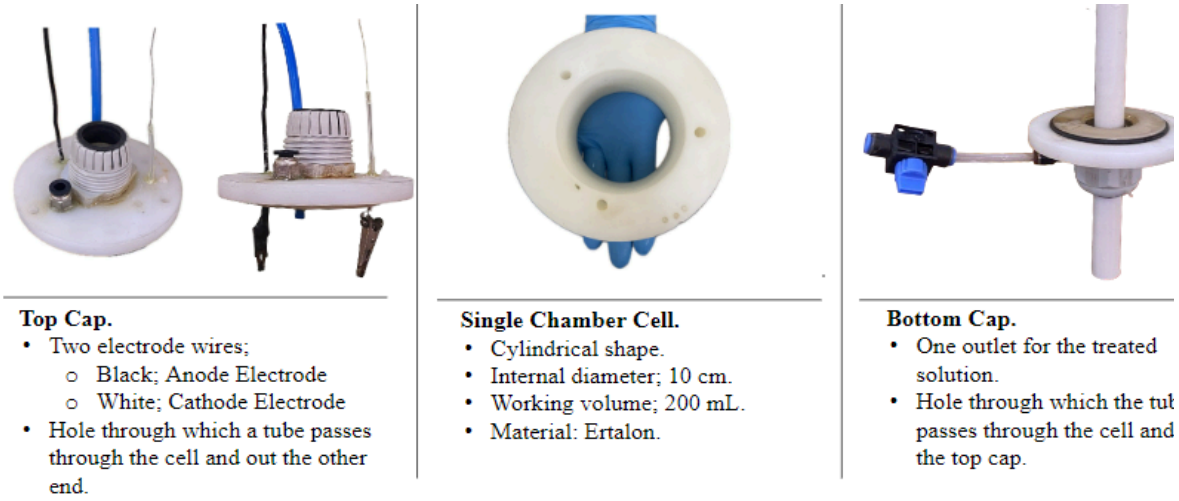


Figure 6: Visualization of the separate components of the Microbial Electrolytic Cell- Reactor of the experimental procedure.

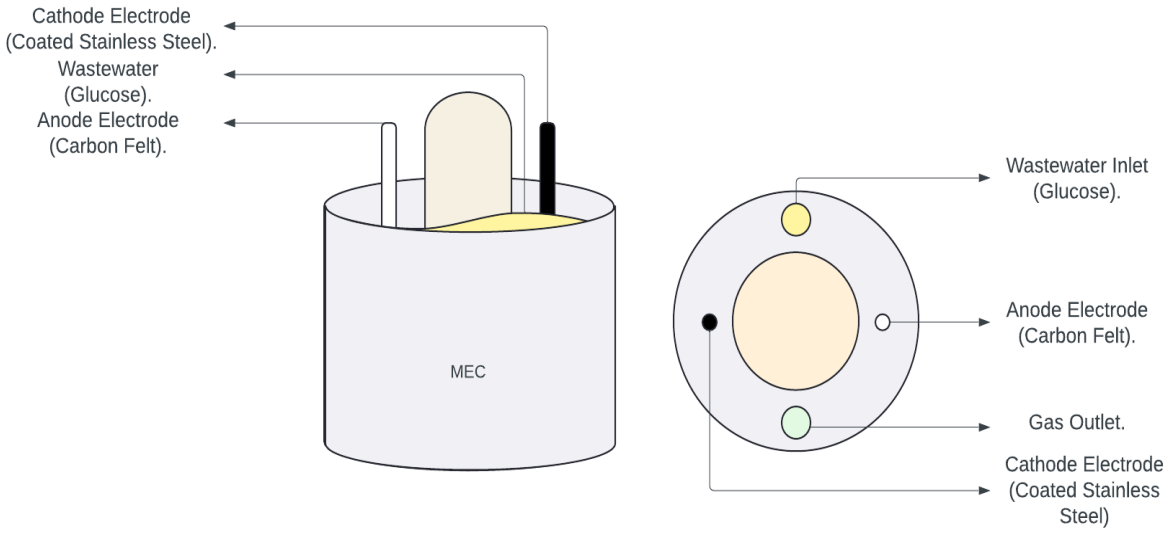


Figure 7: Schematic visualization of Microbial Electrolytic Cell- Reactor.

A very important part of the reactor assembly was ensuring that the chamber was sealed and that anaerobic conditions were imposed, resulting in the effective

operation of the MEC. The reactor was constructed to be gas-tight, preventing the intrusion of air and maintaining the anaerobic environment necessary for microbial activities. Depending on the experimental set, nitrogen (N_2) or carbon dioxide (CO_2) was flushed through the cell until anaerobic conditions were achieved. **Figure 8** illustrates how these conditions were established, by flushing out the air using one of the two gases mentioned. Once the reactor was purged of air, the gas flow was halted, and the reactor was sealed to be gas- tight. This ensured the anaerobic conditions necessary for microbial activity were preserved throughout the experimental process.

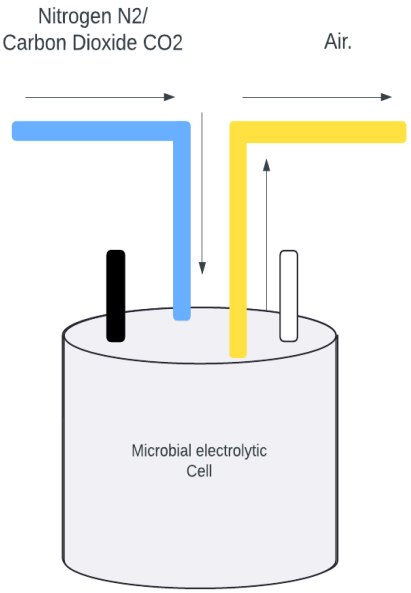


Figure 8: Schematic visualization of Reactor Purge with either Nitrogen (N_2) or Carbon Dioxide (CO_2) before the start of the operation.

The application of voltage between the two electrodes and the observation of electrochemical reactions happening inside the reactor were observed by connecting the electrodes to a Potentiostat SP-150 Biologic which was connected to a PC. Via the Potentiostat the following measurements were taken, which will be analyzed further in the Methods section.

- Open Circuit Voltage Curves: Ewe(V)- Time(sec).
- Constant Voltage Application Curves: I(mA)- Time(sec).

In summary the MEC reactor's design emphasized material properties, structural configuration, and environmental control. The use of Ertalon for the single-chamber cell provided the necessary durability and chemical resistance, while the top and bottom caps facilitated efficient fluid handling and maintenance of anaerobic conditions. The configuration also allowed for easy measurements of pH, COD, and other solution parameters. Additionally, the connection to the potentiostat enabled real-time monitoring of the conditions between the electrodes, enhancing understanding of the reactor's internal processes and contributing to efficient hydrogen production through microbial electrolysis.

4.2 Methods.

The experimental procedure in this thesis is divided into two main parts. The first part involves the modification of the cathodic electrode. The second part focuses on the production of hydrogen (H_2) using the Microbial Electrolysis Cell (MEC), utilizing the modified cathodes from the first part. This structured approach ensures a comprehensive evaluation of both the electrode modification process and the subsequent hydrogen production efficiency in the MEC.

4.2.1 Cathodic Electrode Experimental Procedure.

4.2.1.1 Cathode Electrode Modifications.

Sandblasting has been employed in various studies to enhance the surface properties of anode electrodes in microbial electrolysis cells [[88] 85].

Surface modification was applied using the sandblasting technique available in the equipment of KOS HELLAS. Specifically, the material used for sandblasting consisted of specialized commercial powders of brown aluminum oxide (Al_2O_3) with specific grain sizes as detailed below and in **Table 1**:

- Commercial name (Al_2O_3): F80, Grain Size: 150 - 212 μm.
- Commercial name (Al_2O_3): F120, Grain Size: 90 - 125 μm.
- Commercial name (Al_2O_3): F220, Grain Size: 53 - 75 μm.

[88][] Wang, H., & Ren, Z. J. (2013). A comprehensive review of microbial electrochemical systems as a platform technology. Biotechnology Advances, 31(8), 1796–1807. https://doi.org/10.1016/j.biotechadv.2013.10.001

Table 1: Specialized commercial powders of brown aluminum oxide (Al_2O_3) with specific grain sizes.

Commercial Name(Al_2O_3)	Illustration	Grain Size
F80		150 - 212 μm
F120		90 - 125 μm
F220		53 - 75 μm

These powders were selected to ensure commercial availability for potential scaling-up processes on larger surfaces, particularly on heat exchanger components. The code names of the surfaces (samples) are derived from the commercial names of the powders.

Each stainless-steel sample underwent a pre-treatment process before sandblasting, consisting of the following steps:

- Thorough degreasing with soapy water.
- Rinsing with double-distilled water.
- Rinsing with a mixture of ethanol and acetone (50%-50%).
- Drying in an oven at 50°C for 10 minutes.

The samples were then allowed to return to room temperature inside a desiccator.

The sandblasting process was carried out under specific parameters to ensure the repeatability of the procedure. The distance was maintained at 30 cm from the surface, and the sandblasting duration was kept at 20 seconds. This was established based on visual observation of surface changes in the samples.

4.2.1.2 Cathodic Electrode Analysis.

The characterization of these surfaces included profilometry measurements to characterize and confirm the microscale of the surfaces & SEM imaging.

The profilometry measurements were performed using an AMBIOS Xp-2 profilometer under the following scanning conditions:

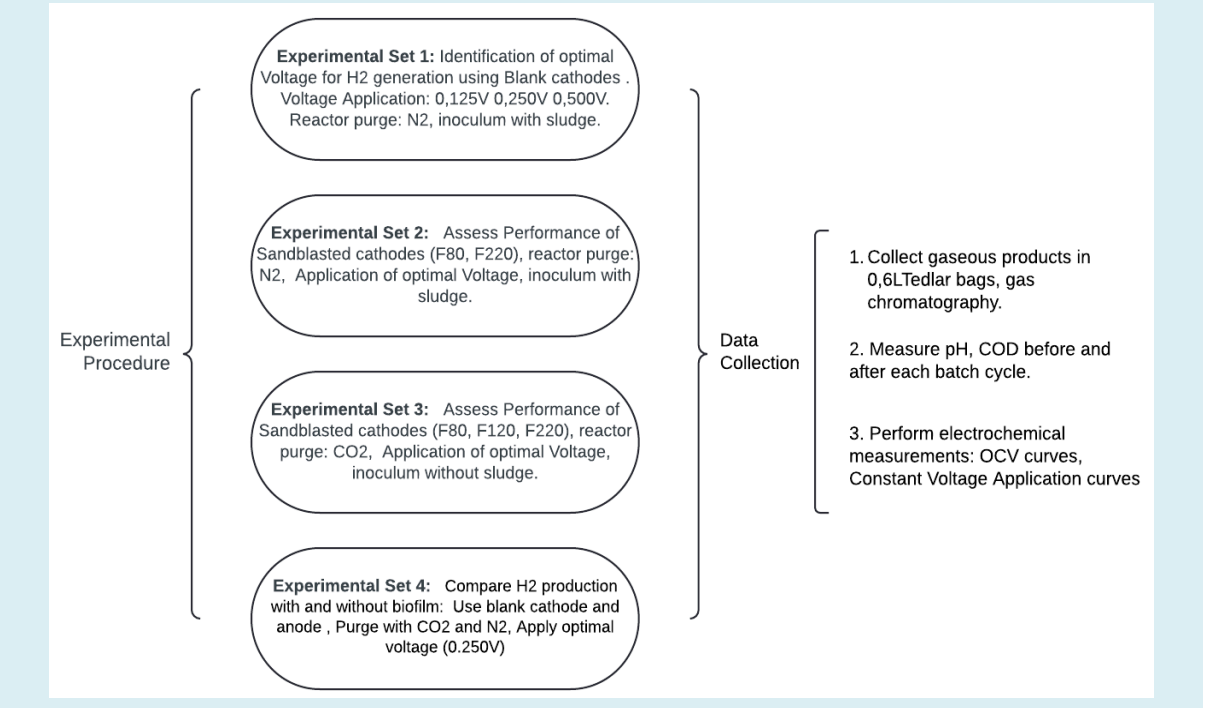
- Speed: 0.15 mm/s
- Scanning length: 3 mm
- Contact force: 1.0 mg
- Scale range: 400 μm

Measurements were taken for all three samples mentioned above, as well as for two samples without topography (blanks) for comparison purposes.

Microscopy analyses were also performed using a Scanning Electron Microscope (SEM) to further evaluate the surface modifications of the exchanger surfaces. The SEM analysis provided detailed images and helped in assessing the microstructural changes on the treated surfaces.

4.2.2 Microbial Electrolysis Cell Experimental Procedure.

The MEC operation involved four experimental rounds, each designed to assess different parameters and conditions, which can be compactly organized in the following **Figure 9** and **Table 2**:



This diagram outlines the experimental procedure and data collection methods used in the MEC studies.

The Experimental Procedure of the MEC operation includes:

- the identification of optimal voltage for H_2 generation,
- assessment of sandblasted cathodes' performance, and
- comparison of hydrogen production with and without biofilm.

Data Collection methods include:

- gas collection and analysis via a Gas Chromatographer with TCD capabilities (equipped with HaySep-Q column)
- pH and COD measurement of the treated solution,
- and electrochemical assessments (OCV & CstV Cues) via a Potentiostat SP-150 Biologic.

Detailed Experimental Procedure for MEC Operation.

The following procedure was consistently followed in every set and experiment to ensure reliability and reproducibility of the results:

1. Preparation of the Solution:
    - Preparation of the solution of choice (Solution 1 or Solution 2 depending on the experimental set) before assembling the reactor.
        - ❖ **Solution 1**: Comprises 75.5 mL buffer (pH 7), 10 mL glucose solution, 2.5 mL sludge, 2 mL KCl solution, and 10 mL electrolyte solution.
        - ❖ **Solution 2**: Contains the same components as Solution 1 but without sludge. This solution is used to assess the impact of sludge on microbial activity and hydrogen production.

2. Electrode Connection:
    - Connection of the anode electrode (carbon felt) to the white wire. This connection ensures that the anode is correctly positioned for optimal electron transfer.
    - Connection of the cathode electrode of choice (blank, F80, F120, or F220) to the black wire. Selecting different cathode types allows the assessment of surface modifications on hydrogen production.

3. Reactor Assembly:
    - Secure attachment of the top cap, reactor body, and bottom cap to prevent leaks when adding the solution.
    - Proper assembly ensures a gas-tight environment necessary for anaerobic conditions.

4. Solution Addition and Circuit Check:
    - Addition of the prepared solution to the reactor, ensuring that the solution is evenly distributed within the reactor chamber.
    - Use of the potentiostat to check the circuit and ensure the voltage is in the millivolt (mV) scale. If the voltage is not within this range, it indicates that the circuit is not closed, and adjustments are needed.

5. Purging the Reactor:
    - Purging of the reactor with either N_2 or CO_2, depending on the experimental set, until anaerobic conditions are established. Typically, 100-150 mL of gas is used to ensure all oxygen is displaced.

- Stopping the gas flow and sealing the reactor to maintain gas-tight conditions. This step is crucial for creating the anaerobic environment required for microbial activity.

6. Electrochemical Measurements:
   - Measurement of Open Circuit Voltage (OCV) Curves: Measuring the potential difference between the anode and cathode without applying external voltage helps assess the thermodynamic efficiency of the cell.
   - Conducting Impedance Spectroscopy: Determining the resistance faced by H+ and e- in moving from the anode to the cathode helps understand the internal resistance and efficiency of the electron transfer process.
   - Measurement of Constant Voltage Application Curves: Measuring the current response over time when a constant voltage is applied to the system helps understand the electrochemical performance and efficiency of hydrogen production under a steady-state voltage application.

7. Batch Cycle and Data Collection:
   - Duration of each batch cycle is approximately 8.5 hours. This duration ensures sufficient time for microbial activity and hydrogen production.
   - Disconnection of the electrodes and collection of about 80 mL of the treated solution from the bottom outlet for COD and pH measurements. These measurements help evaluate the effectiveness of the treatment process.

     Initial Measurements of Starting Solutions:
     - ❖ Solution 1: COD ~ 1000 mg/L, pH ~ 7.
     - ❖ Solution 2: COD ~ 1000 mg/L, pH ~ 7.

8. Reactor Disassembly and Electrode Cleaning.
   - Disassembly of the reactor carefully to avoid any damage to the components.
   - Cleaning of the cathodic electrodes using alcohol, acetone, and soapy water. Thorough cleaning removes any residues that may affect subsequent experiments.

- Allowing the electrodes to dry completely before repeating the experiment. Proper drying ensures accurate and consistent results in subsequent trials.

This detailed procedure ensures a systematic approach to conducting experiments, allowing for accurate and reproducible results in the study of hydrogen production in microbial electrolysis cells (MECs). With the experimental procedure clearly established, we can now introduce the specific experimental sets, detailing the unique parameters and objectives of each. The table below provides a concise overview of these experimental sets, including all relevant specifics, to offer a preliminary understanding of the methodology and goals pursued in each set.

Table 2: Summary of Experimental Sets in Microbial Electrolysis Cell Operation.

Set	Cathode Electorde	Anode Electrode	Reactor Purge Gas	Solution Composition	Replication	Objective
1st	Blank	Acclimated	N_2-Nitrogen	Solution 1	2 per Voltage applied	Identification of optimal voltage for hydrogen generation.
2nd	Blank F80 F220	Acclimated	N_2-Nitrogen	Solution 2	2 per cathode electrode	Assessment of sandblasted cathodes (F80 and F220) performance under N_2 purge.
3rd	Blank F80 F12 F220	Acclimated	Carbon Dioxide-CO_2	Solution 2	2 per cathode electrode	Evaluation of sandblasted cathodes (F80, F120, and F220) performance under CO_2 purge.
4th	Blank	Blank	Carbon Dioxide-CO_2	Solution 2	2	Comparison of hydrogen production with and without biofilm using blank electrodes.

Solution 1: Comprises 75.5 mL buffer (pH 7), 10 mL glucose solution, 2.5 mL sludge, 2 mL KCl solution, and 10 mL electrolyte solution.

Solution 2: Contains the same components as Solution 1 but without sludge. This solution is used to assess the impact of sludge on microbial activity and hydrogen production.

Electrolyte Solution (per 100mL): 2g Na_2CO_3, 2.5g $NaHCO_3$, 2.5g Na_2CO_3, 2.5g Na_2SO_4, 2.5g $K_4P_2O_7$.

Glucose Solution (per 100mL): 1.2g Glucose, 99mL H_2O.

Following the summarized table of experimental sets that were followed, a detailed explanation is provided below to ensure a comprehensive understanding of the study.

First Set of Experiments:

This round aimed to identify the optimal voltage for hydrogen generation using blank (unmodified) cathodes. Voltages of 0.125V, 0.250V, and 0.500V were applied. The reactor was purged with N_2, and the solution contained a small amount of sludge consortia (75.5 mL buffer at pH 7, 10 mL glucose solution, 2.5 mL sludge, 2 mL KCl solution, and 10 mL electrolyte solution).

The sludge consortia were included to determine if the presence of microbes would enhance hydrogen production by increasing the biofilm population on the cathode, thereby potentially increasing the overall microbial activity and hydrogen generation. Each electrode was used twice for each voltage application.

Second Set of Experiments:

This round assessed the performance of sandblasted cathodes (F80 and F220) under N_2 purge conditions. The solution composition was similar to the first round, and each electrode was used twice. The use of N_2 ensured anaerobic conditions necessary for microbial activity, avoiding the inhibitory effects of oxygen on anaerobic microbes.

Third Set of Experiments:

This round evaluated the performance of sandblasted cathodes (F80, F120, and F220) with CO_2 purge. The solution composition was the same as in previous rounds, but

without the sludge. CO_2 was chosen because, in some studies, it has been shown to aid in the breakdown of organic substrates by microbes, though results are mixed. CO_2 also maintains anaerobic conditions needed for microbial processes [89]. Each electrode was used twice.

Fourth Set of Experiments:

This round compared hydrogen production with and without biofilm, using a blank cathode and anode (carbon cloth material with the same size as the cathode). Conditions and solution composition remained similar to previous rounds. Each electrode was used twice.

Data Collection of Experimental Procedure:

Batch cycles lasted approximately 8 hours, which was determined by monitoring the microbial activity under voltage application over time, gaseous products were collected in gas-sampling Tedlar bags with a capacity of 0,600L and analyzed using gas chromatography (GC) with thermal conductivity detector (TCD) capabilities. pH and Chemical Oxygen Demand (COD) measurements were taken before and after each batch cycle. Monitoring pH helps ensure the environment remains conducive to

[89][] Satinover, S. J., Rodriguez, M., Campa, M. F., Hazen, T. C., & Borole, A. P. (2020). Performance and community structure dynamics of microbial electrolysis cells operated on multiple complex feedstocks. Biotechnology for Biofuels, 13(1). https://doi.org/10.1186/s13068-020-01803-y

microbial activity, while COD measurements indicate the extent of organic matter breakdown and treatment efficiency [90 91 92 93 94 95 96].

Electrochemical measurements included:

- Open Circuit Voltage (OCV) curves, which measure the potential difference between the anode and cathode when no external voltage is applied,

[90] Pandis, P. K. et al. Bioenergy Production from Tannery Waste via a Single-Chamber Microbial Fuel Cell with Fly Ash Cathodic Electrodes. Key Engineering Materials 962, 105-112, doi:10.4028/p-0xWsyq (2023).

[91] Pandis, P. K., Georgala, M., Nanou, P. & Stathopoulos, V. N. Fabrication and Study of 3D Printed ABS-Carbon Composite Anodes for Single Chamber Microbial Fuel Cells. Key Engineering Materials 962, 113-120, doi:10.4028/p-3QcQuv (2023).

[92] Savvidou, M. G., Pandis, P. K., Mamma, D., Sourkouni, G. & Argirusis, C. Organic Waste Substrates for Bioenergy Production via Microbial Fuel Cells: A Key Point Review. Energies 15, 5616, doi:10.3390/en15155616 (2022).

[93] Cheng, S., & Logan, B. E. (2011). High hydrogen production rate of microbial electrolysis cell (MEC) with reduced electrode spacing. Bioresource Technology, 102(3), 3571–3574. https://doi.org/10.1016/j.biortech.2010.10.025

[94] Liu, H., & Logan, B. E. (2004). Electricity generation using an Air-Cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environmental Science & Technology, 38(14), 4040–4046. https://doi.org/10.1021/es0499344

[95] Tremouli, A. et al. Operation and Electro(chemical) characterization of a microbial fuel cell stack fed with fermentable household waste extract. Global NEST Journal 21, 253-257, doi:10.30955/gnj.002996 (2019).

[96] Tremouli, A. et al. Performance assessment of a four-air cathode membraneless microbial fuel cell stack for wastewater treatment and energy extraction. E3S Web of Conferences 116, 00093, doi:10.1051/e3sconf/201911600093 (2019).

providing insights into the cell's thermodynamic efficiency. [90 91 92 95 [97] [98] [99] [100]].

- Constant Voltage Application curves: These curves measure the current response over time when a constant voltage is applied to the system. This helps in understanding the electrochemical performance of the cell, the efficiency of hydrogen production, and the impact of different operational parameters [[101]].

[97][] Kamperidis, T., Pandis, P. K., Argirusis, C., Lyberatos, G. & Tremouli, A. Effect of Food Waste Condensate Concentration on the Performance of Microbial Fuel Cells with Different Cathode Assemblies. Sustainability 14, 2625 (2022)

[98][] Wang, H., & Ren, Z. J. (2013). A comprehensive review of microbial electrochemical systems as a platform technology. Biotechnology Advances, 31(8), 1796–1807. https://doi.org/10.1016/j.biotechadv.2013.10.001

[99][] Kadier, A., Chaurasia, A. K., Sapuan, S. M., Ilyas, R. A., Cheng, P., MA, Alabbosh, K. F. S., Rai, P. K., Logroño, W., Hamid, A. A., & Hasan, H. A. (2020). Essential factors for performance improvement and the implementation of microbial electrolysis cells (MECs). In Springer eBooks (pp. 139–168). https://doi.org/10.1007/978-981-15-6872-5_7

[100][] Albuquerque, M. M., Martinez-Burgos, W. J., De Bona Sartor, G., Medeiros, A. B. P., De Carvalho, J. C., & Soccol, C. R. (2024). Microbial electrolysis cells in biohydrogen production. In Biofuel and biorefinery technologies (pp. 429–453). https://doi.org/10.1007/978-3-031-49818-3_17

[101][] Kadier, A., Simayi, Y., Abdeshahian, P., Azman, N. F., Chandrasekhar, K., & Kalil, M. S. (2016). A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alexandria Engineering Journal, 55(1), 427-443. doi:10.1016/j.aej.2015.10.008

5. Results.

5.1 Cathode Modification Results.

5.1.1 Profilometry.

The profilometry analysis of the sandblasted stainless-steel samples provides valuable insights into the surface roughness induced by different particle sizes of Al_2O_3. The key parameter of interest, Ra (average roughness), was measured along with Rq (root mean square roughness) and Rp (maximum peak height). The results are presented below in diagrams, with the horizontal axis representing the scanning length and the vertical axis representing the topography range in micrometers for each modified surface.

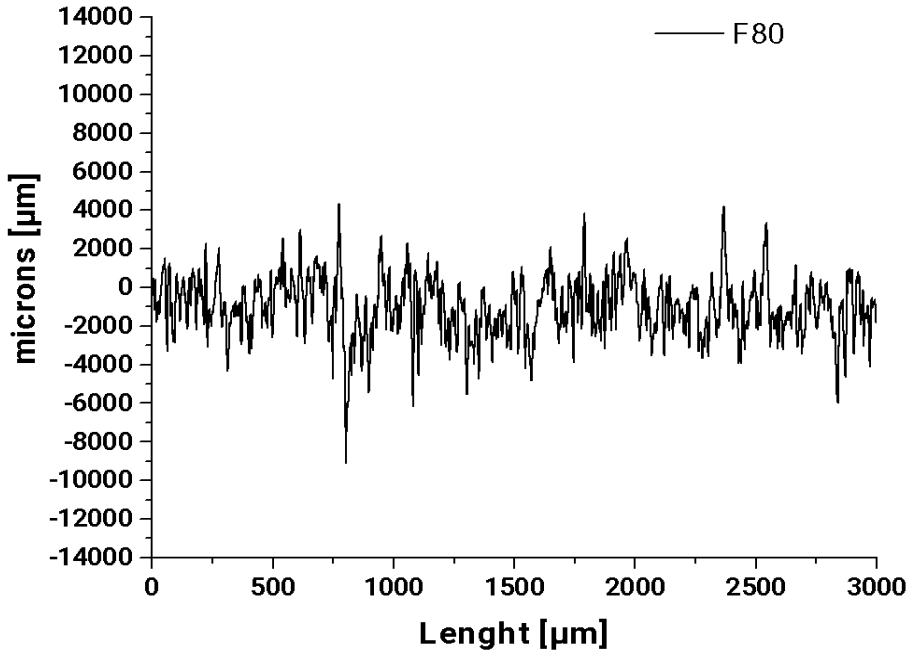


Figure 10: Scanning Length vs. Topography Range for sandblasted cathodic electrode F80.

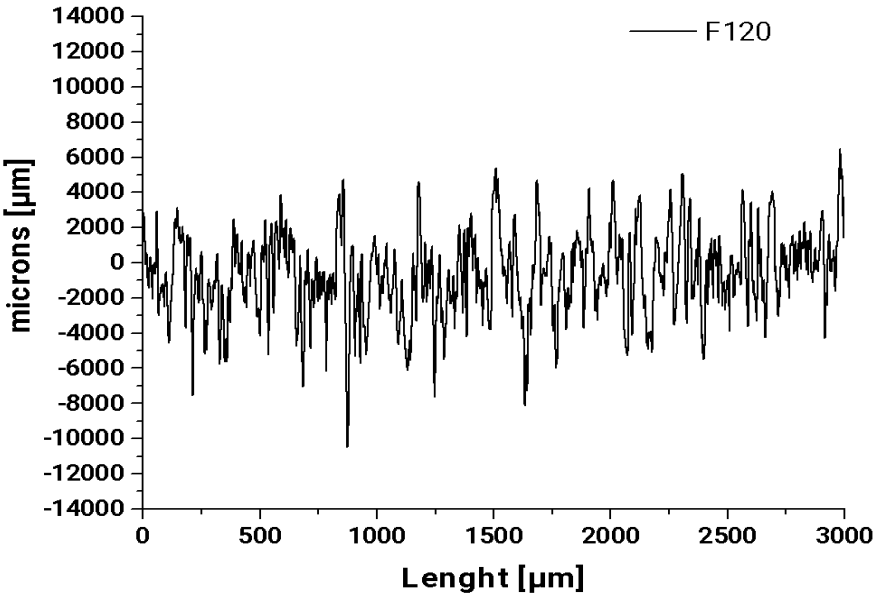


Figure 11 : Scanning Length vs. Topography Range for sandblasted cathodic electrode F120.

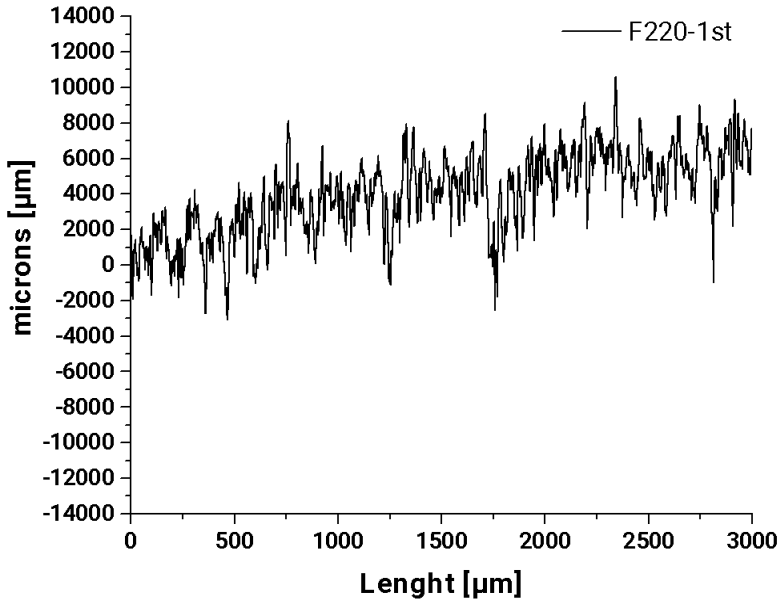


Figure 12: Scanning Length vs. Topography Range for sandblasted cathodic electrode F220.

Based on the parameter calculations using the instrument's software, all surface-related parameters were computed, with the most significant parameter

being the surface roughness Ra, expressed in nanometers (nm). Additionally, a graphical representation of the results from **Table 3** is provided in **Figures 10, 11, 12.**

Table 3:surface-related parameters computed, expressed in nanometers (nm)

Sample	Ra [nm]	Rq [nm]	Rp [nm]	Z(M)-Z(R) [nm]
Bank	250.3	333.8	735.7	-939.7
F80	1228.4	1567.3	5254.3	-1895.0
F120	1908.6	2444.6	5767.5	-5464.7
F220	1835.1	2256.3	7002.5	-8416.5

F80 Sample.

The F80 sample exhibited a moderate level of surface roughness, with an Ra value of 1228.4 nm. The particle size range (150 - 212 μm) used for sandblasting created noticeable depressions and protrusions, but the overall texture was relatively uniform. This level of roughness is indicative of effective sandblasting, where the particles were able to adequately scrape the surface, but not to the extent seen in smaller particle sizes.

F120 Sample.

The F120 sample showed the highest surface roughness, with an Ra value of 1908.6 nm. The particle size range (90 - 125 μm) proved to be optimal for creating significant surface disruptions. The SEM images supported these findings, revealing depressions and protrusions. The increased roughness suggests that F120 particles have an ideal balance of size and impact energy, maximizing surface area and creating a highly irregular topography. This characteristic is particularly advantageous for applications requiring extensive surface modification.

F220 Sample.

The F220 sample, with an Ra value of 1835.1 nm, also displayed considerable roughness, though slightly less than F120. The finer particle size range (53 - 75 μm) resulted in a surface texture that, while rough, was more uniform compared to F120. The peaks and valleys were smaller, indicating that while the surface was effectively altered, the impact was not as substantial as that achieved with the F120 particles. This finer roughness may be suitable for applications that require moderate surface disruption without extreme topographical changes.

Comparative Analysis.

The F120 sample stands out as having the highest roughness, which can be attributed to the optimal particle size for achieving significant surface modification. The F80 and F220 samples, while also rough, do not reach the same level of topographical alteration as F120.

5.1.2 Scanning Electron Microscope Imaging.

The surface morphology of sandblasted stainless steel (SS304) samples, modified using Al2O3 powders of different particle sizes- (F80 (150 - 212 μm), F120 (90 - 125 μm), and F220 (53 - 75 μm)-, was analyzed using Scanning Electron Microscopy (SEM). Below, the SEM images at magnifications ranging from 50x to 800x are presented, enabling the identification of depressions and protrusions in the surface topography. This detailed analysis facilitates a comprehensive comparison of the surface alterations among the samples, highlighting the variations in roughness and topographical features resulting from the sandblasting process.

F80 Sample.

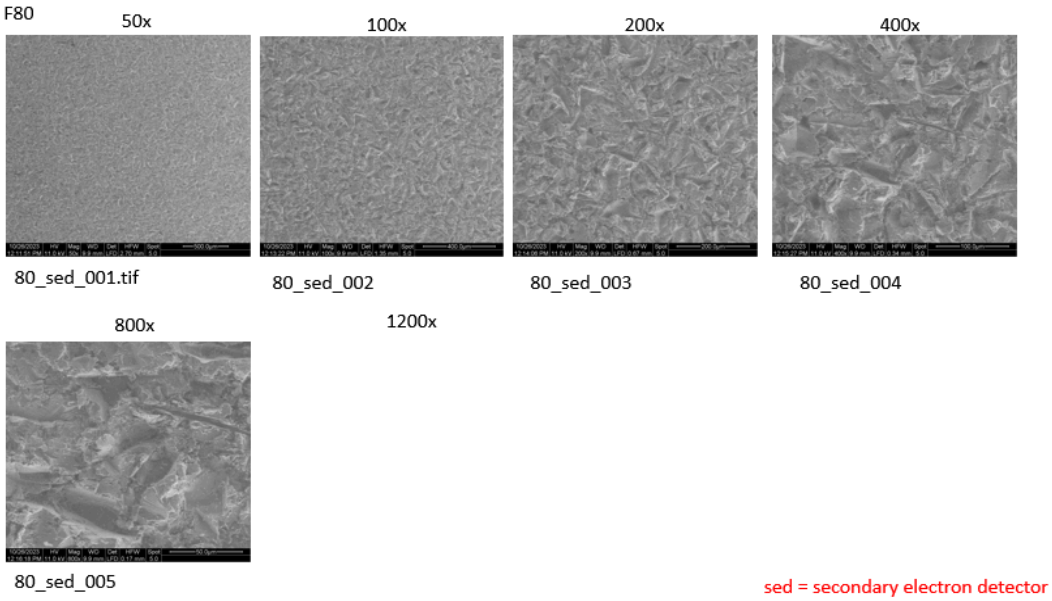


Figure 13: Surface Morphology of F80 Sandblasted Stainless Steel at Various Magnifications (50x to 800x).

F120 Sample.

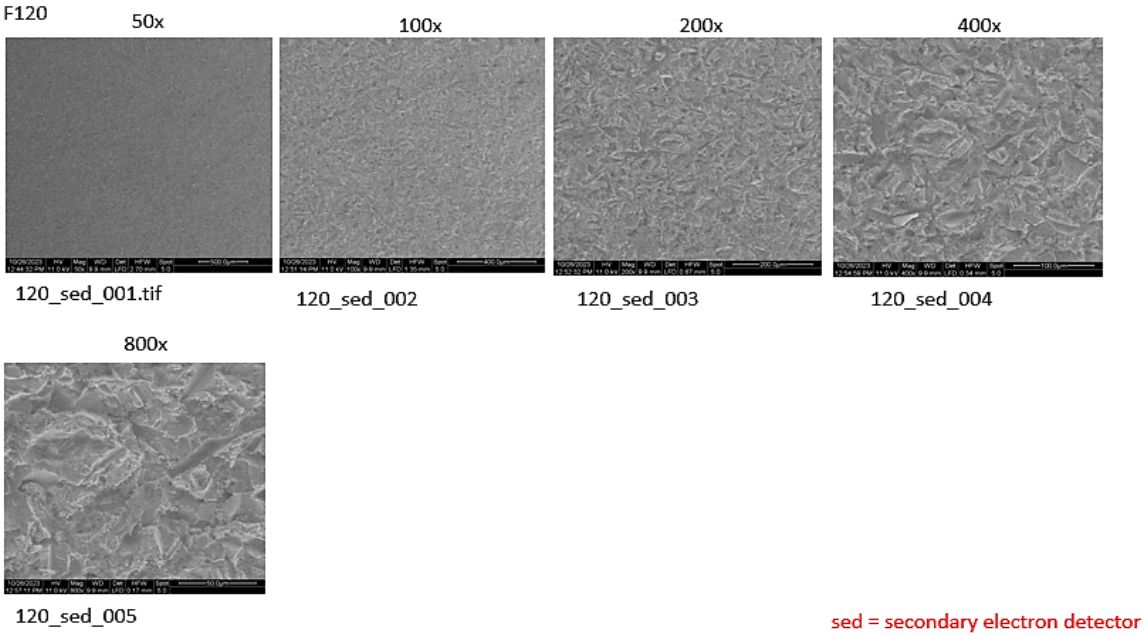


Figure 14: Surface Morphology of F120 Sandblasted Stainless Steel at Various Magnifications (50x to 800x).

F220 Sample.

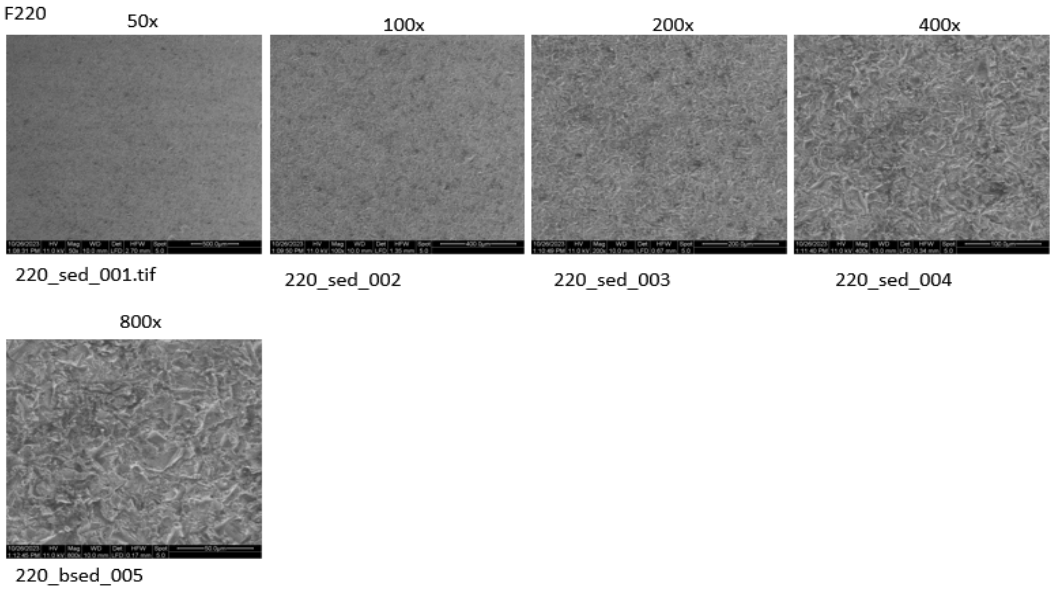


Figure 15 : Surface Morphology of F220 Sandblasted Stainless Steel at Various Magnifications (50x to 800x).

The SEM images of the F80 sample show a moderately rough surface with visible projections and cavities. The surface texture appears relatively uniform, with the sandblasting process creating consistent, even though moderate, topographical changes. The "peaks" and dents in this sample are distinguishable but not as pronounced as in the F120 sample. The uniform pattern suggests effective sandblasting, but the alterations are not as extensive as those seen in F120.

The SEM images of the F120 sample exhibit the most pronounced surface roughness among the samples analyzed. The images reveal deep intrusions and high peaks, indicating significant surface alteration. The topographical changes are highly irregular, with substantial variations in height and depth. This irregular texture is a result of the optimal particle size range (90 - 125 μm) used in the sandblasting process, which effectively disrupts the surface to create a highly roughened texture.

The enhanced roughness provides a greater surface area for applications such as catalytic reactions and microbial attachment in microbial electrolysis cells (MECs).

The F220 sample shows a rough surface, but the texture is finer, and more uniform compared to the F120 sample. The SEM images reveal smaller depressions and protrusions, indicating that the smaller particle size (53 - 75 μm) creates less pronounced surface features. While the surface is still altered, the extent of the topographical changes is less significant than in the F120 sample. This finer texture may be suitable for applications requiring moderate surface roughness without extreme alterations.

Comparison and Analysis.

The SEM imaging results indicate that the F120 sample underwent the most substantial surface modifications. The particle size range used for F120 strikes a balance between sufficient impact energy to create significant surface disruptions and optimal coverage to ensure uniform roughness. In contrast, the F80 and F220 samples exhibit less pronounced topographical changes, with F80 showing moderate roughness and F220 displaying a finer, more uniform texture.

5.2 Microbial Electrolytic Cell Operation Results.

5.2.1 Electrochemical Results.

The Current (I) in Electron Transfer in MECs.
In microbial electrolysis cells (MECs), the measured current (I) is indicative of the rate of electron transfer from the anode to the cathode. Higher current generally corresponds to a higher rate of electron transfer, which is crucial for electrochemical processes such as the Hydrogen Evolution Reaction (HER). This correlation is supported by several studies. According to Pawar et al. the current generated in MECs is a direct result of extracellular electron transfer (EET) mechanisms where electrons are transported from electroactive bacteria to the electrode surface [[102]].

[102][] Pawar, A. A., Karthic, A., Lee, S., Pandit, S., & Jung, S. P. (2020). Microbial electrolysis cells for electromethanogenesis: Materials, configurations and

What is more, increased current signifies enhanced electrochemical activity, suggesting more efficient electron flow through the external circuit, which is essential for processes like hydrogen production [100].

Constant Voltage Application Curves:

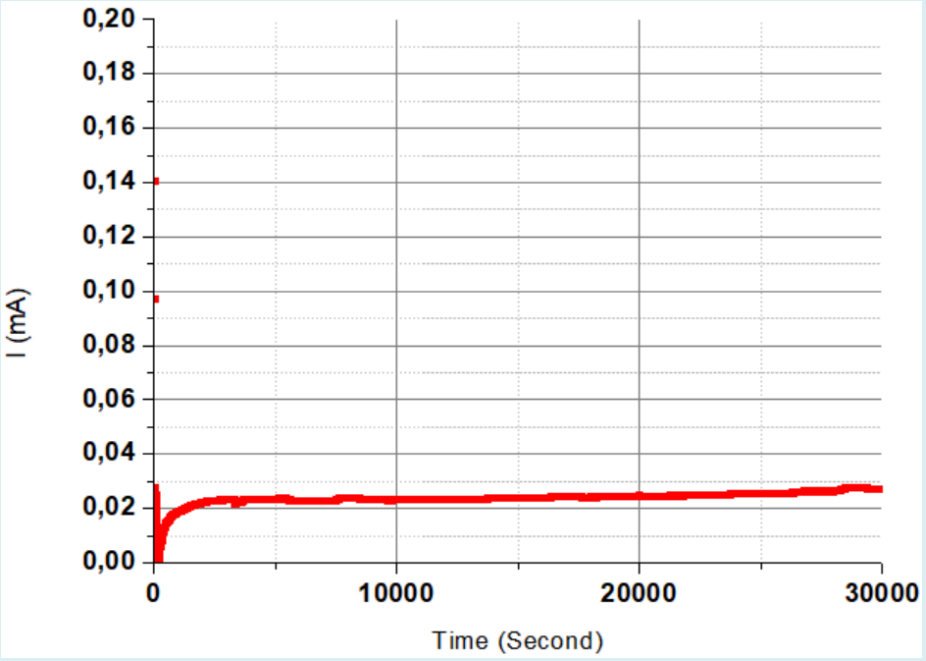
The current response at different voltages reveals insights into the electron transfer dynamics. The blank cathode's performance varies significantly with the applied voltage, indicating how voltage influences electron movement and subsequent hydrogen production.

5.2.1.1 1st Set of Experiments: Blank Cathode (0.125V, 0.25V, 0.50V):

In this round the objective was to identify the optimal voltage for hydrogen generation using blank (unmodified) cathodes. Voltages of 0.125V, 0.250V, and 0.500V were applied. The reactor was purged with Nitrogen (N_2), and the solution contained a small amount of sludge consortia (75.5 mL buffer at pH 7, 10 mL glucose solution, 2.5 mL sludge, 2 mL KCl solution, and 10 mL electrolyte solution).

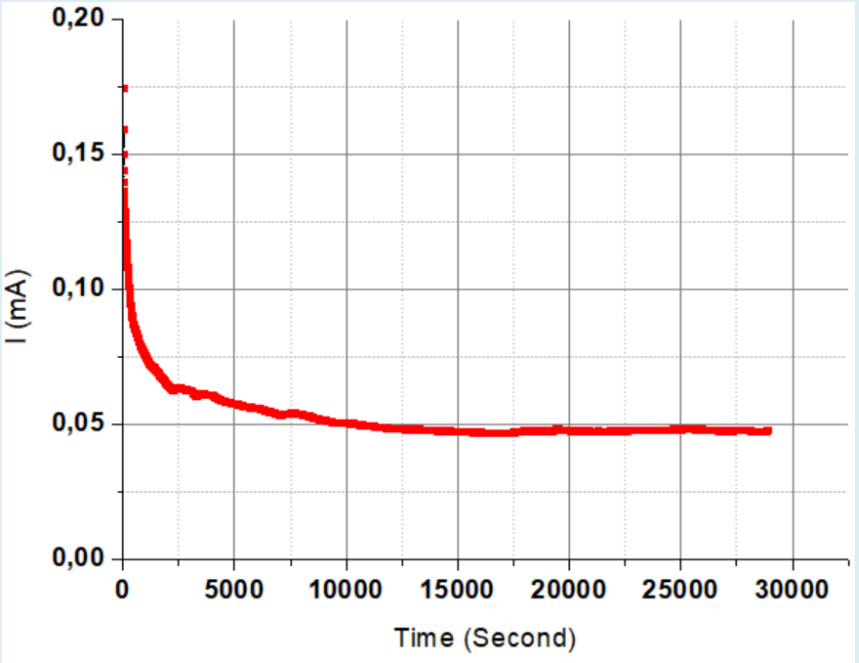
1st Experiment: 0,125V Application.

operations. *Environmental Engineering Research/Enviromental Engineering Research*, *27*(1), 200484–0. https://doi.org/10.4491/eer.2020.484



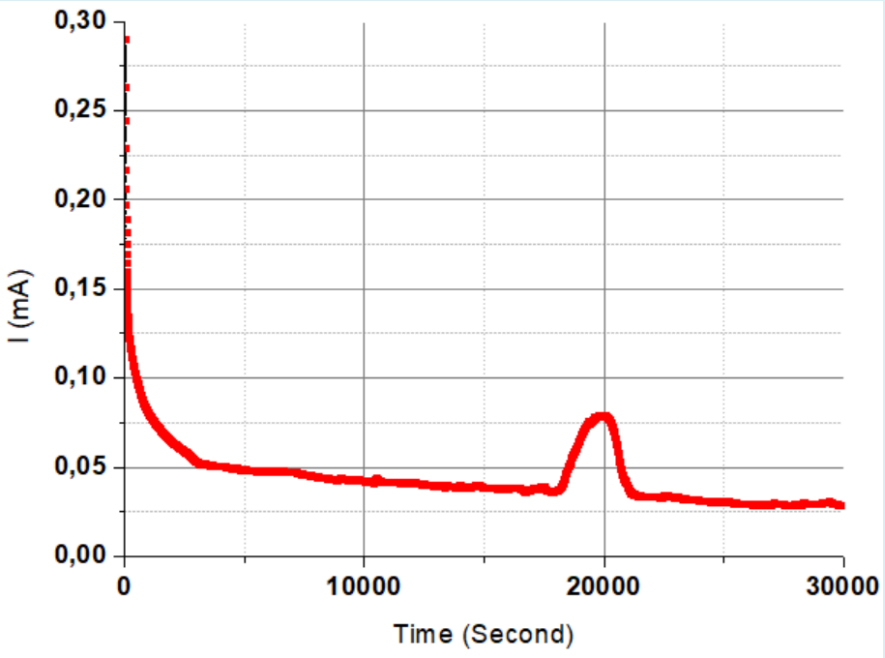
Graph 1: Blank Cathode- @0.125V- N_2 Purge.

2nd Experiment: 0.250V Application.



Graph 2: Diagram: Blank Cathode- @0.250V- N_2 Purge.

3rd Experiment: 0.500V Application.



Graph 3: Blank Cathode- @0.500V- N_2 Purge.

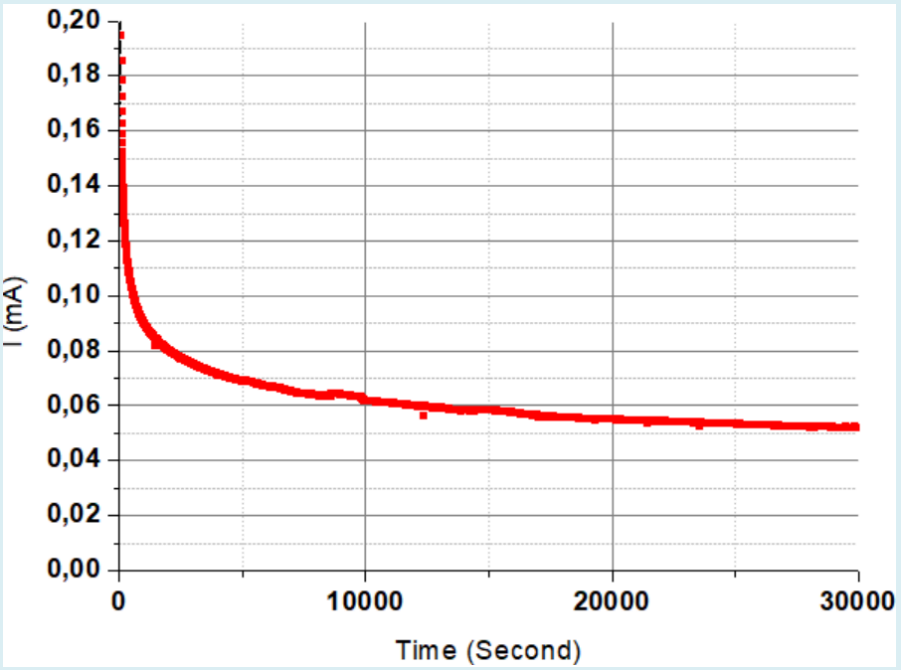
At 0.125V, the current is relatively low, suggesting limited electron transfer. This could be due to the insufficient driving force for moving electrons from the anode to the cathode, highlighting the need for a higher applied voltage to enhance electrochemical reactions.

The current peaks at 0.250V indicating the most efficient electron transfer. This voltage appears to strike a balance, providing enough energy to overcome activation barriers without causing excessive resistance or inefficiencies. The optimal current at this voltage suggests it is the most effective for promoting hydrogen evolution, aligning with the gas chromatography results showing the highest hydrogen production at this voltage.

At 0.500V, the current decreases despite the higher applied voltage. This anomaly could be due to increased resistance to electron transfer, as suggested by impedance spectroscopy. The higher voltage might introduce additional barriers or inefficiencies, limiting the overall electron transfer rate and reducing the current. This observation indicates a threshold voltage beyond which the efficiency of electron transfer decreases, emphasizing the importance of optimizing the applied voltage for maximum efficiency.

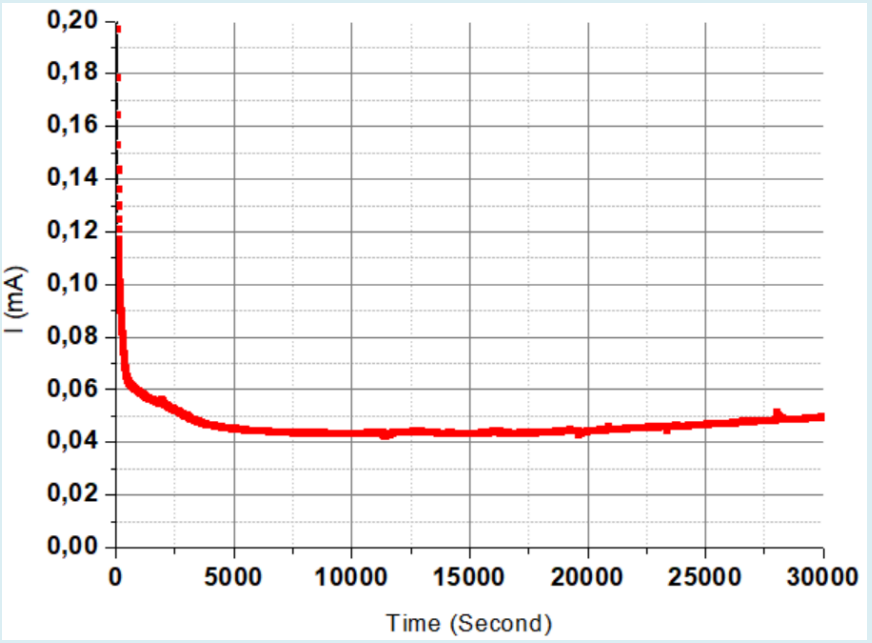
5.2.1.2 2nd Set of Experiments: (Different Cathodes, N_2, Sludge, @0.250V).

1st Experiment: F80 Cathode (0.250V).



Graph 4: F80 Cathode- @ 0.250V - N_2 purge.

2nd Experiment: F220 Cathode (0.250V).



Graph 5: F220 Cathode- @ 0.250V- N_2 Purge.

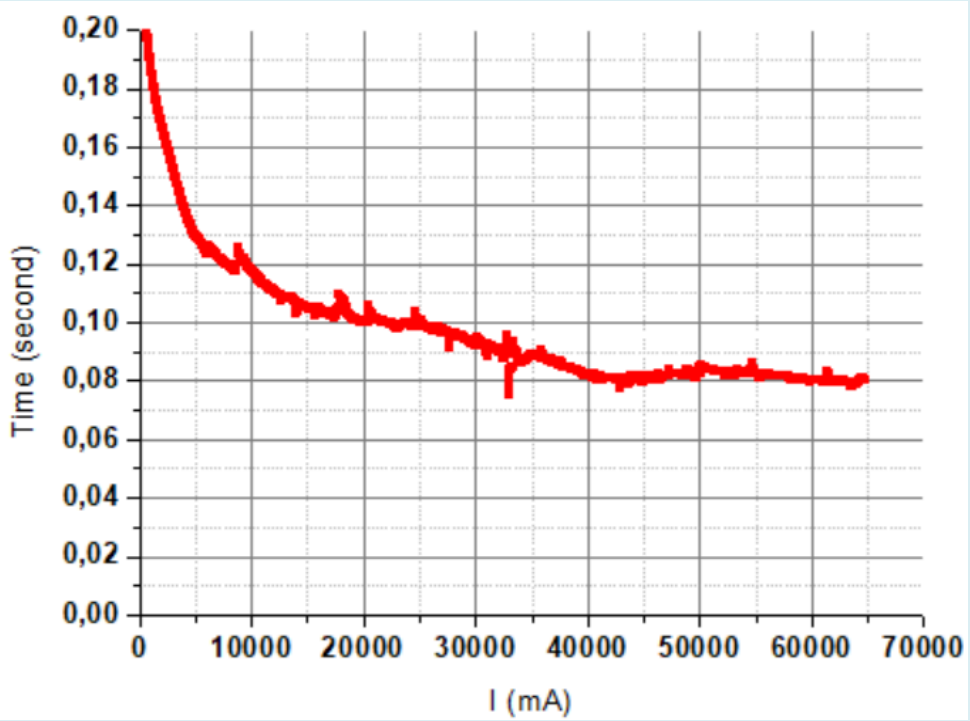
The F80 cathode exhibits a significant current response, suggesting a decent rate of electron transfer. The stability of the current indicates efficient electron transfer dynamics.

The F220 cathode demonstrates a significant current response as well with decent electron transfer being measured. The current stability exhibited in **Graphs 5 & 6** is encouraging suggesting that there is efficient electron transfer dynamics between the microbes, the anode and the cathode electrode.

There is on significant difference between F80, F220, and the blank cathode in the I(mA) production, which would indicate that the electron transfer is about the same.

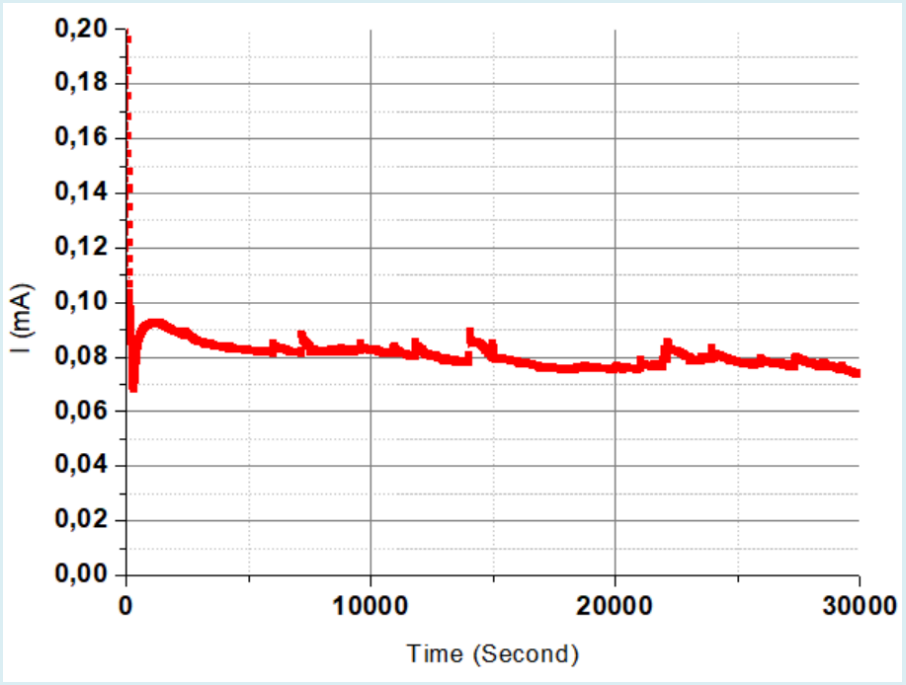
5.2.1.3 3rd Set of Experiments: Different Cathodes (CO_2, No Sludge)

1st Experiment: Blank Cathode (0.25V), in a CO_2 environment.



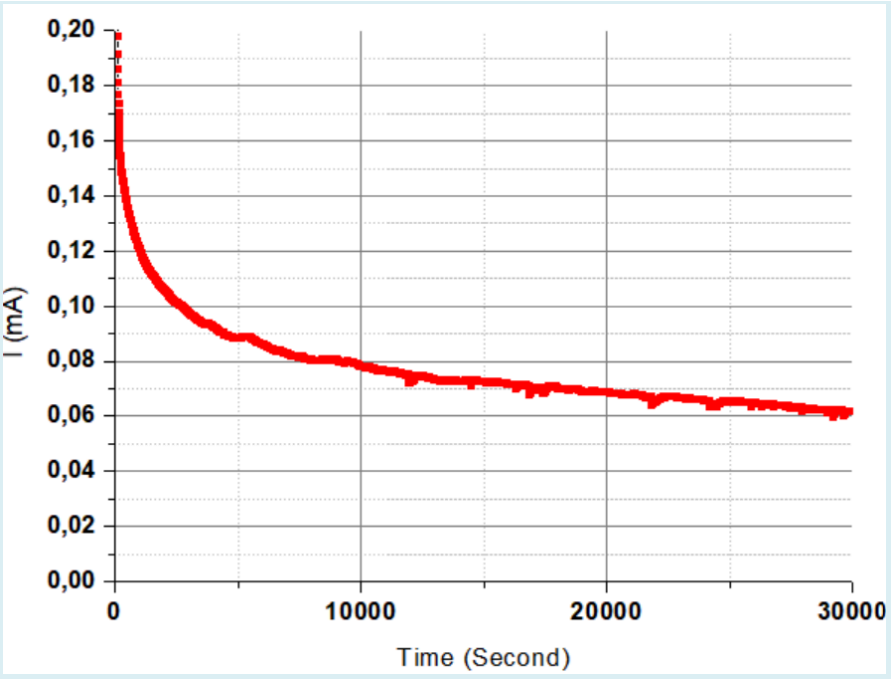
Graph 6: Diagram: Blank Cathode- @0.250V – CO_2 Purge.

2nd Experiment: F80 Cathode (0.25V), in a CO_2 environment.

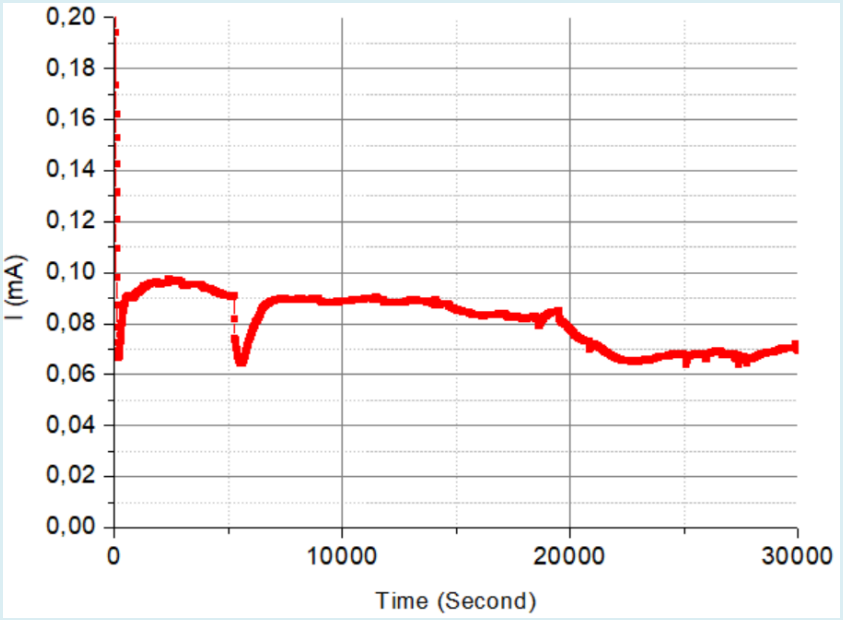


Graph 7: F80 Cathode- @0.250V - CO_2 Purge.

3rd Experiment: F120 Cathode (0.250V), in a CO_2 environment.



Graph 8: F120 Cathode- @ 0.250V- CO_2 Purge.



Graph 9: F220 Cathode- @0.250- CO_2 Purge.

The current response of the blank cathode in a CO_2 environment, is about doubled to its performance with N_2 purge. The consistent current suggests stable electron transfer capabilities, indicating a consistent catalytic activity for hydrogen production.

Again, similarly to the blank cathode, the current response of the F80 cathode in a CO_2 environment, is about doubled to its performance with N_2 purge. When using the F120, the current response is very similar to the response of the system when using the blank and F80 cathodes. Finally, the current response of the F220 cathode in a CO_2 environment is again about doubled to its performance with N_2 purge.

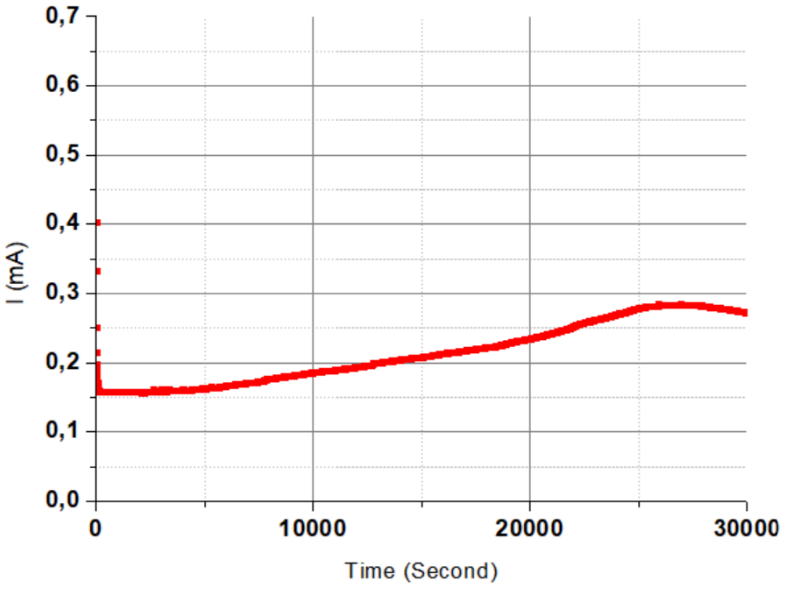
The CO_2 environment demonstrated a positive impact on electron transfer, consistent with previous research indicating that CO_2 aids in the breakdown of organic matter by microbes, thereby enhancing electron production [[103]]. Among the various cathodes tested, there were minimal differences in current. This observation

[103][] Quraishi, M., Wani, K., Pandit, S., Gupta, P. K., Rai, A. K., Lahiri, D., Jadhav, D. A., Ray, R. R., Jung, S. P., Thakur, V. K., & Prasad, R. (2021). Valorisation of CO2 into Value-Added Products via Microbial Electrosynthesis (MES) and Electro-Fermentation Technology. Fermentation, 7(4), 291. https://doi.org/10.3390/fermentation7040291

is understandable, as the cathodes are composed of the same material and the surface morphology of the cathode does not directly influence electron production. Electron generation occurs at the anode, where it is significantly influenced by microbial activity and the applied voltage, rather than the cathode surface. This highlights the role of the anode and environmental conditions in determining electron production efficiency.

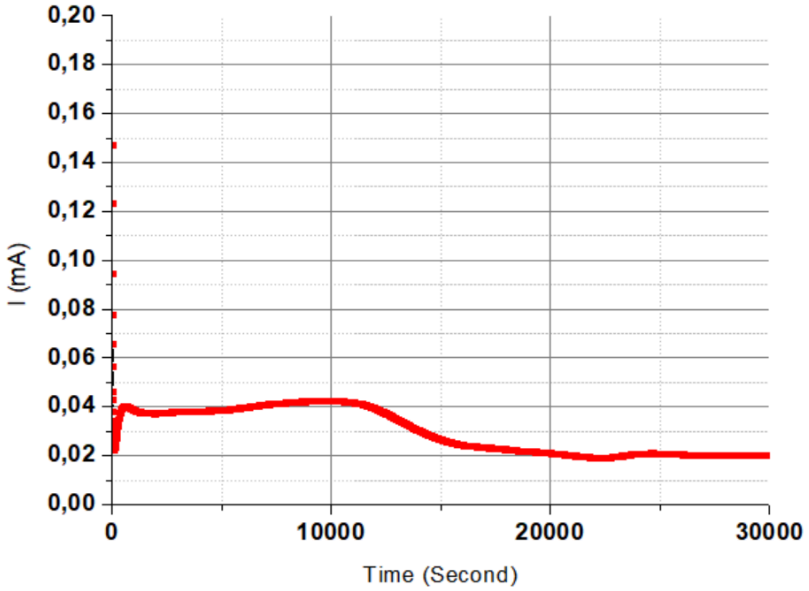
5.2.1.4 4th Set of Experiments: Blanks (No Microbes on the Anode), CO_2 & N_2 environment.

1st Experiment: Blanks with CO_2 (0.25V):



Graph 10: Blank Anode (Carbon Cloth) and Blank Cathode- @0.250V – CO_2 Purge.

2nd Experiment: Blanks with CO_2 (0.25V):



Graph 11: Blank Anode (Carbon Cloth) and Blank Cathode- @0.250V – CO_2 Purge.

The current response for the blank cathodes in a CO_2 environment is notably higher compared to the anode with microbes. This interesting observation suggests that CO_2 is being reduced to CO within the reactor, resulting in **Graph 11**.
The current response for the blank cathodes in an N_2 environment is low, as seen in **Graph 12,** indicating minimal electron transfer. The absence of microbial activity and catalytic surfaces limits electron transfer and subsequent hydrogen production. The difference between N_2 and CO_2 environments highlights the potential role of CO_2 in facilitating electron transfer and enhancing current.

5.2.2 Gas Chromatography (GC) Results.

1st Set of MEC experiments.

Determining the Optimal Voltage for Maximum Hydrogen Production.

The initial experiment aimed to identify the optimal voltage for hydrogen production using a blank stainless-steel cathode. The voltages tested were 0.125V, 0.250V, and 0.500V. The results are summarized in the following **Table 5** as follows:

Table 4: First Set- Determining the Optimal Voltage for Maximum Hydrogen Production.

Voltage (V)	Cathode Electrode	H2 Sample (% v/v)	H_2 (mL)	CH_4 (mL)	Conductivity (mS/cm)	COD (mg/L)	pH

0,125	Blank	0.02	0.12	0.06	18.3	582	6.84
0,250	Blank	0.03	0.18	0.06	20.1	567	6.97
0,500	Blank	0.01	0.06	0.06	20.9	593	6.92

- The optimum voltage was set at 0.250 V, resulting in the highest concentration of hydrogen in the reactor, 0.18 mL of H_2.
- Methanogenesis, the production of methane by microorganisms, is a common effect observed in microbial electrolysis cells (MECs). Many studies have reported this phenomenon, particularly when using complex substrates like glucose in the presence of sludge, which contains methanogenic archaea [104]. These microbes play a crucial role in converting organic compounds into methane under anaerobic conditions.

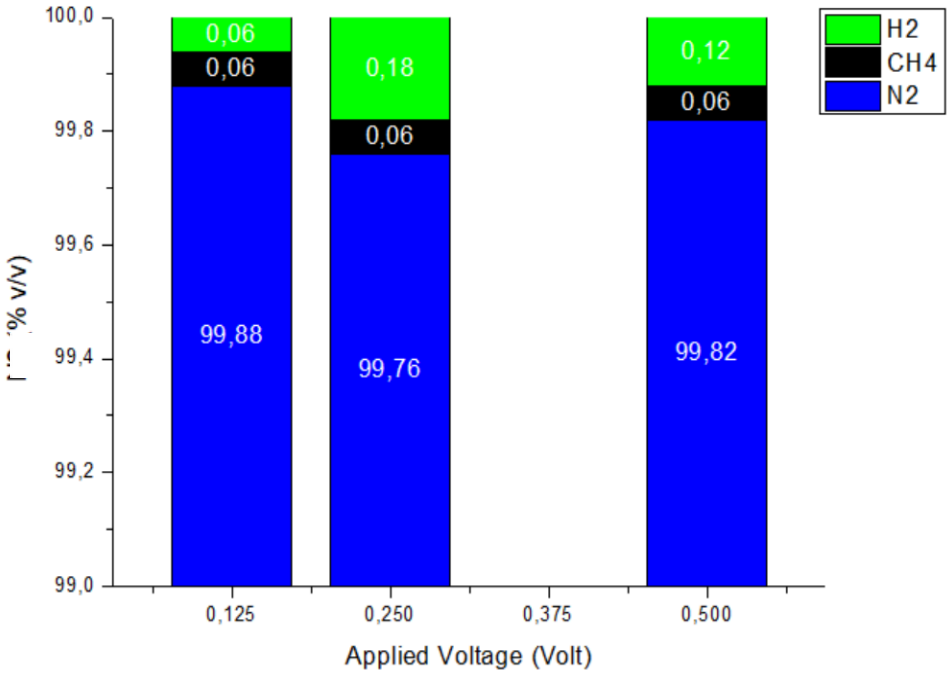


Figure 16: Gas Composition for the First Set of Measurements.

[104][] Wu, Q., Xiao, H., Zhu, H., Pan, F., & Lu, F. (2023). Carbon Felt Composite Electrode Plates Promote Methanogenesis through Microbial Electrolytic Cells. Energies, 16(11), 4416. https://doi.org/10.3390/en16114416

- Chemical Oxygen Demand (COD): The COD values indicate the amount of organic matter present in the solution. The COD decreased from an initial value of 1000 mg/L to values between 567 mg/L and 593 mg/L, which indicates that there was microbial activity and substrate consumption during the experiments.
- pH: The pH values remained relatively stable, ranging from 6.84 to 6.97. This stability suggests that the buffer solution effectively maintained a conducive environment for microbial activity and electrochemical reactions.
- Conductivity: Conductivity measurements varied slightly, with values from 18.3 to 20.9 mS/cm. This indicates the ionic strength and overall conductivity of the solution, which is crucial for efficient electron transfer and microbial metabolism.

2nd Set of MEC Experiments:

Performance with Different Cathodes (N_2, Sludge).

Following the identification of the optimal voltage, the experiment proceeded with different sandblasted cathodes (F80 and F220) under the same conditions (0.250V, N_2 purge, and sludge):

Table 5: Second Round- Results with N_2 Purge and Sludge for Different Cathodes.

Voltage (V)	Cathode Electrode	H_2 Sample (% v/v)	H_2 (mL)	CH_4 (mL)	Conductivity (mS/cm)	COD (mg/L)	pH
0.250	Blank	0.03	0.18	0.06	18.3	582	6.84
0.250	F80	0.05	0.30	0.06	20.8	402	6.81
0.250	F220	0.07	0.42	0.06	20.0	356	6.78

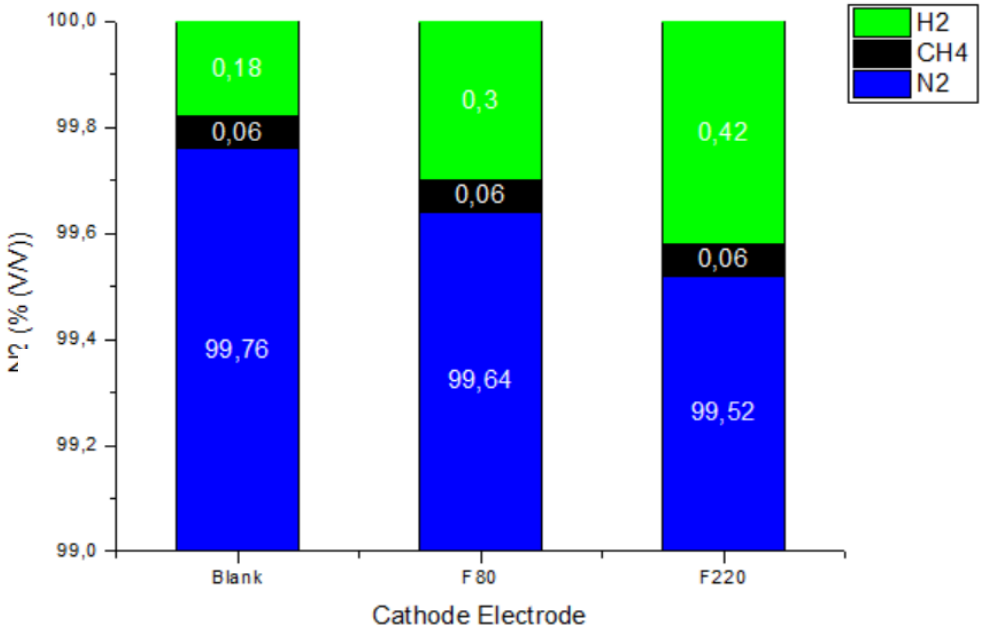


Figure 17: Gas Composition for the Second Set of Measurements.

- Comparing the second-round results with the blank cathode at 0.25V from the first-round significant increases in hydrogen production can be spotted. The F80 cathode showed a hydrogen production increase from 0.18% to 0.30%, a 66.67% rise. The F220 cathode demonstrated an even more substantial increase, from 0.18% to 0.42%, marking a 133.33% rise. These findings indicate that the sandblasted cathodes (F80 and F220) significantly enhance hydrogen production compared to the unmodified blank cathode under the same voltage application. The F220 cathode produced the highest hydrogen concentration (0.42mL), indicating that the finer particle size resulted in a more favorable surface for hydrogen evolution.
- In these experiments, methanogenesis was consistently observed in the first and second rounds when sludge was present in the added solution. Despite varying the voltage application, the amount of methane produced remained relatively constant. This suggests that the presence of sludge, rather than the applied voltage, is the primary factor driving methane production.

  The presence of methanogenic archaea in the sludge is likely responsible for this effect. These microorganisms utilize the organic substrates provided (such as glucose) and produce. The constant methane production across

different voltages indicates that the methanogenic activity was primarily influenced by the availability of organic substrates and microbial populations in the solution rather than the electrochemical conditions alone.

Studies have demonstrated that integrating microbial electrolysis cells with anaerobic digestion systems using waste-activated sludge (WAS) enhances methane production. For instance, research found that introducing electrodes into a continuous stirring tank reactor with WAS, resulted in a 1.5-fold increase in cumulative methane production [[105]]. The findings from our experiments align with previous research, confirming that the addition of sludge significantly contributes to methane production in MECs.

- Chemical Oxygen Demand (COD): In the second round of experiments, the COD values further decreased to between 356 mg/L and 402 mg/L. This significant reduction indicates enhanced microbial activity and greater substrate utilization, likely due to the improved surface properties of the sandblasted cathodes (F80 and F220).
- pH: The pH values in this round ranged from 6.78 to 6.81. The slight decrease compared to the first round suggests increased microbial activity and metabolic byproducts, but still within a range that supports efficient microbial and electrochemical processes.
- Conductivity: Conductivity measurements for this round were slightly higher, ranging from 20.0 to 20.8 mS/cm. The increased conductivity could be because of the release of ions from the enhanced microbial degradation of organic matter.

3rd Set of MEC Experiments:

GC Results with CO_2 Purge and No Sludge for Different Cathodes.

To evaluate the performance without microbial influence and test the influence carbon dioxide can have on Hydrogen production, the reactor was purged with CO_2 and sludge was not added. All the sandblasted cathodes (F80, F120, F220) as well as the blank cathode were tested at the optimal voltage of 0,250V:

[105][] He, W., Zhang, D., Zhang, L., Ai, Z., Guo, Z., Yang, T., Zhai, L., & Huang, C. (2024). Enhanced Methanogenesis of Waste-Activated Sludge (WAS) in a Continuous Stirring Tank Reactor with Stealth Electrodes. Fermentation, 10(3), 158. https://doi.org/10.3390/fermentation10030158

Table 6: Third Set- Results with CO_2 Purge, no Sludge for Different Cathodes.							
Voltage (V)	**Cathode Electrode**	**H_2 Sample (% v/v)**	**H_2 (mL)**	**CH_4 (mL)**	**Conductivity (mS/cm)**	**COD (mg/L)**	**pH**
0.250	Blank	0.06	0.36	0.00	19.6	545	6.75
0.250	F80	0.08	0.48	0.00	21.8	348	6.57
0.250	F120	0.09	0.54	0.00	19.8	330	6.50
0.250	F220	0.12	0.72	0.00	19.6	321	6.46

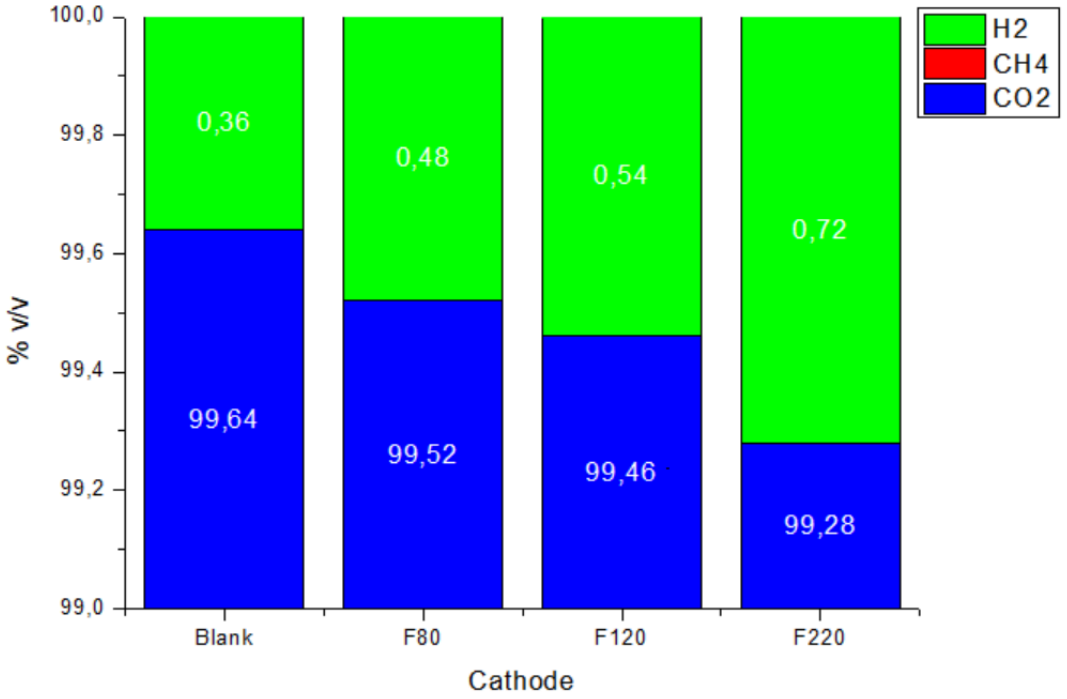


Figure 18: Gas composition for the third set of Measurements.

The F220 cathode again produced the highest hydrogen concentration (0.72mL), further validating its effectiveness.

- In the third set of experiments, the reactor was purged with CO_2 rather than N_2 and operated without sludge. This setup had a significant positive effect on hydrogen production. The hydrogen concentration increased notably when comparing the results with those from the first round (which used sludge and N_2 purge). For instance, using the F220 cathode, the hydrogen production increased from 0.07% to 0.12%, representing a 71% increase. Similarly, the F80 cathode saw an increase from 0.05% to 0.08%, a 60% rise.

These results indicate that CO_2 purge, in the absence of sludge, enhances hydrogen production efficiency in the microbial electrolysis cells.

- No methanogenic effect was observed. This lack of methane, combined with the exclusion of sludge in the solution, supports our previous statement that potentially the methanogenic activity observed in the first and second rounds was primarily due to the presence of sludge. The consistent methane production in earlier rounds, despite different voltage applications, indicates that the sludge was the primary factor driving methanogenesis.
- Chemical Oxygen Demand (COD): The third round showed further decreases in COD, with values between 318 mg/L and 348 mg/L. The lower COD values indicate effective organic matter degradation, with CO_2 purging potentially boosting hydrogen production and solution treatment.
- pH: The pH values in this round were slightly lower, ranging from 6.44 to 6.57. The decrease in pH reflects the acidic byproducts of microbial metabolism.
- Conductivity: Conductivity ranged from 19.6 to 22.4 mS/cm. The higher conductivity in this round suggests increased ionic concentration due to more extensive organic matter breakdown and electrochemical reactions facilitated by the CO_2 purging.

4th Set of Experiments: Blank Anode and Cathode Tests.

Finally, the reactor was tested using both blank anode and cathode under CO_2 and N_2 purge conditions, to compare hydrogen production with and without biofilm using blank electrodes.

Table 7: Fourth Round- Blank Anode and Cathode Tests.

Voltage (V)	Anode Electrode	Cathode Electrode	Gas Purge	H_2 Sample (% v/v)	CO (mL)	Conductivity (mS/cm)	COD (mg/L)	pH
0.250	Blank	Blank	CO_2	0.00	0.00	19.7	879	6.98
0.250	Blank	Blank	N_2	0.00	0.12	20.0	882	6.96

Carbon Monoxide (CO) Production: In the experiment with CO_2 purge, a small amount of carbon monoxide (0.02%) was detected. This could be attributed to the electrochemical reduction of CO_2. The reduction of CO_2 can occur at the cathode, especially under specific conditions, leading to the formation of carbon monoxide. The reaction can be represented as:

$$CO_2 + 2H^+ + 2e^- \rightarrow CO + H_2O \quad (7)$$

This reaction is more likely to occur when CO_2 is used as the purge gas, providing a source of CO_2 for reduction. The negligible hydrogen production in the absence of sludge further emphasizes the role of microbial activity in enhancing hydrogen production. Microbial biofilms facilitate the breakdown of organic matter (glucose), the transfer of electrons and protons, thus leading to the hydrogen evolution reaction. In the absence of these biofilms, the electrochemical activity is significantly reduced.

Concluding, the results from the blank anode and cathode experiments indicate that the presence of biofilm and surface modification are crucial for efficient hydrogen production in MECs. The detection of carbon monoxide in the CO_2 purged system suggests possible electrochemical reduction of CO_2, which warrants further investigation. These findings underscore the importance of optimizing both the microbial and electrochemical aspects of MECs to maximize hydrogen production efficiency.

6. Discussion & Summary of Findings.

Based on the results from the first set of experiments, where the highest hydrogen production was observed at 0.250V, subsequent experiments consistently applied this voltage to optimize hydrogen production.

The decision to stop using sludge in the solution was based on the observation that its presence led to steady methane (CH_4) production, which was undesirable. Methane production consumes hydrogen and has a lower energy value compared to hydrogen [[106]]. In fact, the energy content of methane CH_4 is approximately 55.5 MJ/kg, whereas hydrogen has a higher energy content of 142 MJ/kg. Removing sludge from the solution prevents the formation of methane, thus ensuring that maximizing hydrogen yield was the focus of this thesis.

Based on the results from the third set of experiments (with CO_2 Purge and No Sludge for Different Cathodes), despite observing similar current responses when using different cathodes, in the case of the F220 cathode, a significantly higher hydrogen production was noted in comparison to the other cathodes. Additionally, SEM observations showed that the surface of F120 was much more inconsistent in comparison to that of the F220 electrode. Since the F220 had a higher H_2 production, this finding suggests that the surface of the F120 possibly introduces more hindering mechanisms to hydrogen production. The F220 cathode's superiority in hydrogen production can be attributed to various factors. According to Powar et al. while higher current indicates more electrons available for reduction reactions at the cathode, the overall hydrogen production is determined by other factors as well, such as electrode surface properties, catalytic efficiency, and gas diffusion [102].

1. **Surface Area and Active Sites:**

[106][] Kanellos, G., Monokrousou, E., Tremouli, A., & Lyberatos, G. (2024). Treatment of two-phase olive mill waste and degradation of phenolic compounds with simultaneous bio-electrochemical conversion of CO2 to CH4 using a microbial electrolysis cell. *Sustainable Chemistry and Pharmacy*, *39*, 101589. https://doi.org/10.1016/j.scp.2024.101589

Although the F220 cathode did not have the roughest surface, its topography provides more effective active sites for HER compared to F120. The more consistent surface morphology in F220 could facilitate better catalytic activity. The SEM imaging of the F120 cathode revealed more intense protrusions compared to the F220. While this might suggest a larger surface area, it does not necessarily translate to more effective active sites for the HER to occur. The excessive roughness can create a more complex surface, making it harder for H^+ ions to enter the catalytic site efficiently. This may explain why F120 does not produce as much hydrogen as the F220 cathode despite its higher surface roughness.

2. **Surface Composition and Catalytic Activity:**

The F220 cathode might utilize transferred electrons more effectively for hydrogen production due to its catalytic properties, even if it did not show the highest current. It is a possibility, that the surface of the F220 cathode strikes the optimal balance of exposed elements (Ni, Fe, Cr) in comparison to the other electrodes, which favors the Hydrogen Evolution Reaction (HER). Specifically stainless steel is commonly used for cathodes, and typically contains these elements, which are known for their catalytic properties. nickel (Ni) enhances HER due to its excellent catalytic activity [102], while iron (Fe) contributes to the structural stability and can also act as a catalytic site, although less efficiently than nickel [102]. Finally, chromium is corrosion resistant, which is crucial for the durability of the electrode since it is constantly exposed to solutions that are to be treated.

It is suggested that different surface exposure is caused by the sandblasting process. It could be a possibility that the sandblasting process exposed more nickel on the F220 surface compared to the other cathodes. Given nickel's superior catalytic properties for HER, this increased exposure would enhance the efficiency of hydrogen production on the F220 cathode.

This efficiency can also be attributed to the synergistic effects of the elements present in the alloy, enhancing the overall catalytic activity [[107]]. For example, the combination of Ni and Cr can create a more stable and active surface which can support efficient electron transfer and hydrogen production [107].

3. **Gas Diffusion:**

   Effective gas diffusion is crucial to maintaining active catalytic sites and ensuring continuous hydrogen evolution without the hindrance of gas bubble formation [96]. The design and surface properties of the F220 cathode may support better gas diffusion, preventing hydrogen bubble accumulation and enhancing HER. The more intense surface unevenness on the F120 surface could impede efficient gas diffusion. Hydrogen bubbles formed on these rough surfaces may become trapped in the deeper indentations and prevented from dispersing away from the catalytic site. This accumulation of hydrogen bubbles can slow down the reaction, reducing the effective surface area available for the HER and reducing hydrogen production.

The combined analysis of gas chromatography and electrochemical results reveals the importance of optimizing environmental conditions (gas purging, sludge inoculum, batch cycle, voltage application) and electrode properties (surface modifications, material selection) for efficient hydrogen production in microbial electrolysis cells (MECs). The CO_2 environment greatly enhances the organic matter breakdown from the exoelectrogens, leading to enhanced electron transfer, while the excellent surface structure and catalytic properties of the F220 cathode facilitate efficient hydrogen evolution. These results highlight the need for a comprehensive approach to in electrode analysis and characterization and careful selection of control procedures to maximize MEC activity.

Summary of Findings

- **Methanogenesis:** The presence of sludge in the solution added to the reactor lead to the production of methane CH_4, while the applied voltage played no role in the production of CH_4.
- **Optimal Voltage:** 0.250V was identified as the optimal voltage for hydrogen production, foe this thesis' biofilm.

[107][] Falch, A., & Babu, S. P. (2021). A review and perspective on electrocatalysts containing CR for Alkaline water electrolysis: Hydrogen Evolution Reaction. *Electrocatalysis*, *12*(2), 104–116. https://doi.org/10.1007/s12678-020-00634-7

- **Cathode Performance:** Sandblasted cathodes, especially F220, significantly improved hydrogen yield.
- **CO_2 Purge:** Enhanced hydrogen production efficiency.
- **Sludge in inoculum:** in the absence of sludge the methane CH_4 production was limited.
- **Biofilm Role:** The presence of biofilm is crucial for efficient hydrogen production in MECs.
- **CO Production:** Detected during CO_2 purging, using blank anode and cathode, suggesting electrochemical reduction of CO_2.

7. Future Analysis of Cathodes.

Based on the results obtained, it is clear that further analysis of the cathodes is necessary to better understand their performance and to optimize their properties for enhanced hydrogen production. In this line of thinking some suggestions for further investigation of the cathodic electrodes are elemental analysis with X-Ray Photoelectron Spectroscopy (XPS), surface morphology and topography with Atomic Force Microscopy (AFM) which will assist in understanding the surface of the cathodes and Impedance spectroscopy analysis, which focuses on the electron transfer from the anode to the cathode. In detail:

1. **Elemental analysis with X-ray Photoelectron Spectroscopy (XPS):**
   X-Ray Photoelectron Spectroscopy (XPS) is a technique used to measure elemental composition, chemical state, and electronic state of elements within materials [108 109]. XPS would be a valuable method to perform an elemental analysis of the cathode surfaces, to further understand the surface chemical composition of each cathode electrode being assessed in this study (F80, F120, F220). Additionally, the oxidation states of the elements present on the surface of the cathodes after sandblasting can be observed. Understanding the exact elemental composition and distribution can help in identifying the catalytic sites and their efficiency in the Hydrogen Evolution Reaction (HER).
2. **Surface morphology and topography with Atomic Force Microscopy (AFM):**
   As previously discussed, with the sandblasting technique the surface of the cathodes is altered, resulting in dents, which possibly increase the overall

[108][] Berg, A., & Keim, E. (1993). X-ray Photoelectron Spectroscopy (XPS). https://doi.org/10.1515/iupac.71.0758.

[109][] Lefebvre, J., Galli, F., Bianchi, C., Patience, G., & Boffito, D. (2019). Experimental methods in chemical engineering: X-ray photoelectron spectroscopy-XPS. *The Canadian Journal of Chemical Engineering*. https://doi.org/10.1002/CJCE.23530.

surface of the electrode. To calculate the new surface area and analyze the surface morphology after sandblasting, Atomic Force Microscopy (AFM) can be employed. AFM provides high-resolution images of the surface topography, allowing for precise measurement of surface roughness and the identification of dents. This information is crucial in correlating the surface features of each electrode with their catalytic performance.

3. **Impedance spectroscopy analysis:**
   Impedance spectroscopy analysis has been used in the past in many research articles on MFC (Microbial Fuel Cells) and MECs (Microbial Electrolytic Cells), since it is an efficient and non-destructive test for analyzing MFCs or MECs, helping optimize their construction and compositions to overcome low power generation [[110]]. Analyzing the data from impedance spectroscopy would be beneficial to understand the resistance encountered in electron transfer using the different cathodes. Impedance spectroscopy can help in recognizing and minimizing the various resistances within the MEC system, such as ohmic resistance, charge transfer resistance, and mass transport resistance [[111]]. By comparing these resistances across different cathodes, we can identify which modifications lead to reduced resistance and improved electron transfer efficiency.

[110][] Wang, H., Long, X., Sun, Y., Wang, D., Wang, Z., Meng, H., Jiang, C., Dong, W., & Lu, N. (2022). Electrochemical impedance spectroscopy applied to microbial fuel cells: A review. *Frontiers in Microbiology*, 13. https://doi.org/10.3389/fmicb.2022.973501.

[111][] He, Z., & Mansfeld, F. (2009). Exploring the use of electrochemical impedance spectroscopy (EIS) in microbial fuel cell studies. *Energy and Environmental Science*, 2, 215-219. https://doi.org/10.1039/B814914C.

8. Bibliography